THERAPEUTICAL INVESTIGATIONS ON INDUCED CANCER IN MICE

Effect of the Treatment with Lead Trypan Blue, Thorium Trypan Blue, and Castration on Cancer Induced by Tar and Dibenzpyrene

By

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INAUGURAL DISSERTATION

By due permission of the most experienced Medical Faculty of the University of Uppsala, to be publicly discussed in the Theatre of the Pharmacological Institute, on May 24th, 1948, at 10 o'clock a. m., for the degree of doctor of medicine

By

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Med. Lic., Gtbg

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From the Pharmacological Institute of the University of Uppsala, Sweden.
Head: Professor E. Louis Backman.

Therapeutical Investigations on Induced Cancer in Mice.

Effects of the treatment with lead trypan blue, thorium trypan blue, and castration on cancer induced by tar and dibenzpyrene.

By

Svante Bursell.

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Preface.

I wish to give my most sincerest thanks to Professor NILS GELLERSTEDT, who has been my teacher since I began my studies at the Pathological Institute in Uppsala, for all he has taught me, for all the interest shown me and help given with this and other investigations and not least for his personal friendship. His assistance and encouragement have been invaluable. I should like to add that it was during the years as assistant at the Pathological Institute that I became interested in cancer investigations.

The present investigation has been carried out at the Pharmacological Institute in Uppsala. It gives me the utmost pleasure to be able to thank the Head of the Institute Professor E. LOUIS BACKMAN for all good advice and information which he gave me at the beginning of this work. I should also like to express my thanks for his personal interest and help during the passed years.

I wish to convey my gratitude to my present Chief Professor C. O. NYLÉN for the interest he showed my work and the support he gave me making it possible to complete this investigation.

When the first positive results could be seen in this investigation I consulted the Head of the Race Biological Institute in Uppsala Professor GUNNAR DAHLBERG. I therefore wish to express my thanks to him for his valuable advice and assistance and also for his personal kindness and constructive criticism during the course of the work. The statistical treatment was partly carried out by the Institute and in connection with this I should like to express my gratitude to Mrs. BUME for her thorough and devoted aid. I should also like to thank the other members of the Institute.

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The pharmaceutical firm ASTRA has kindly put gratuitous preparations at my disposition for this I should like to thank them.

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Uppsala, May 1948.

S. Bursell.

CHAPTER I.

Survey of Literature.

Since the end of the 19th century, intensive work has been carried out in order to analyse the origin and development of malignant tumours. The aim was to find a starting point for a successful treatment for this serious illness. The first successful experiments of tumour transplantation, which were first carried out by JENSEN and then by EHRLICH and his co-operators, BASHFORD, LEWIN and others constituted the beginning to the gradually increasing experimental tumour research. FIBIGER stimulated the interest through his experiments on a type of ventricular cancer in rats (1907). If the rat ate a certain cockroach (Periplaneta americana), that had in its muscles a parasite (Spiroptera neoplastica or Gongylonema neoplasticum) it then penetrated into the ventricular epitela. This parasite, which is a kind of roundworm, produced a cancer in up to 50 per cent of the rats; it continued to grow even after the parasite had disappeared. It had been observed that workers in certain occupations, such as chimney sweeps, analine workers, certain miners etc. had a higher incidence of cancer. The Japanese, YAMAGIWA and ICHIKAWA produced cancer by painting the external ear of rabbits (1915). TSUTSUI obtained cancer by painting the backs of mice with tar (1918). After this progress, one attempted to isolate and — if possible — produce synthetically those carcinogenic agents which must be found in coal tar. The work was arduous because it appeared that only tar from certain kinds of coal contained larger amounts of carcinogenic agents. By isolating the distillation fractions at temperatures above 300°—400°, it was possible to obtain them highly concentrated. The first agent with strong carcinogenic effect was found by KENNAWAY (1929): 1, 2, 5, 6 — dibenzanthracene. Later, COOK & HEWETT & HIEGER isolated the first carcinogenic agent from coal tar: 1, 2 — dibenzpyrene. Finally one has, particularly during recent years, worked on spontaneously occuring mammary cancer in certain mice strains. As early as 1907, MURRAY could establish a certain inheritance of this tumour. BITTNER's finding of the so-called milk factor is of special interest. In these mice, with a high frequency of spontaneous mammary cancer, there is found a factor in the milk which is of great importance to the origin of mammary cancer. BITTNER has during recent years reported that there is much in favour of these milk factors being caused by virus. The tumour tissue can be dried, kept frozen for a few years and then induced with a positive result. It is probable, apart from certain conditions, that in animals this virus is also needed in order to develop mammary cancer. In England they have had animals from American strains which have regularly developed mammary cancer for 20 years. With no provable cause, this capacity in the mice strain to develop spontaneous mammary

cancer has disappeared (verbal information from Sir Guy to Prof. E. Louis Backman)

It was first through the arise of experimental cancer research that it became possible to perform therapeutic experiments.

TORRES (1904) was the first to recommend the application of colloidal metals when treating malignant tumours. After some sporadic experiments, the interest in this possibility was renewed by v. WASSERMANN and KEYSSER (1911). Their aim was to try to treat the tumour cells therapeutically without injuring the other cells of the body in animals bearing cancer. This could be achieved by introducing agents with tumour-affinity into the blood vessels. They examined above all selenium and tellurium, and decided to employ selenium-eosin as being less toxic. Intravenous injections first caused a softening, and then a liquefaction of the tumour. They could not establish the recidive. When examining animals with tumours in the softening stage, they were able to establish that the tumour was intensely red against the grey, or only slightly red surroundings. Simultaneously NEUBERG & CASPARI & LÖHE (1912) worked with, among other things, heavy metals and tried to produce tumour-affinity compounds which would increase the autolyse in the tumour. Above all they examined colloidal preparations of zinc, lead, arsenic, antimony, vanadium, mercury, copper, cobalt, silver, gold, platinum, iridium, rhutenium, osmium, palladium, and rhodium among others. Encouraging results were obtained with lead, copper and especially with cobalt and silver. It was soon found, however, that the therapeutic dosage lay so near the lethal one that the method could not be applied to human beings. Other investigators also reached the same conclusion. This was the situation when BELL, through results of his treatment of trophoblast with colloidal lead preparations, was encouraged to continue treating other malignant tumours also.

In order to facilitate the survey of that literature which concerns more closely the author's investigations, it is tabulated giving the most important data of each work, and a summary after each section of the table. Even though this table cannot claim to be complete, the majority of works in this field should, however, be found in it. By the term "tumour" is meant malignant tumours unless the contrary is stated.

In connection with these tables, it must be emphasized that the results of experiments with animals cannot, as a matter of course, be considered as applicable to human beings. This self-evident reservation is particularly true where mortality is concerned, for one may suspect that animals' power of resistance to lead poisoning, or poisoning with other salts of metals differs from that of man. Furthermore, it must be pointed out that the experiments carried out are often made on small material. Occasionally the authors have not stated how comprehensive their material was, and then one is apt to presume that it was not very large. Because of this the tabulated account is divided into two parts, one comprising material

A. Treatment of Tumours with Metals.

1. Lead.

Author	Size and type of material	Methods of treatment	Results
	Experimental investigations with smaller sets.		
Neuberg & Caspari 1912	mice with transplanted tumour 14	lead	uncertain effect
Neuberg, Caspari & Löhe 1912	mice with transplanted tumour. (Rats with transpl. tumour, number not stated)	lead	uncertain effect
Krause 1929	mice with transplanted tumour	org. lead preparations	certain prep. inhibit occurrence of the tumour, or cause recurrence after some time
Levi 1929	mice with transplanted tumour	colloidal lead prep.	no effect
Levi & Volta 1930	mice with transplanted tumour (number not stated)	colloidal lead with zinc sulphate	no effect
v. Hevesy & Wagner 1930	mice with transplanted tumour	injections of lead nitrate	no selective accumulation of lead in tumour tissues
Vecchi 1930	rats with transplanted tumour 24	Dezanis' lead prep.	treatment gives no change in length of life
Suzuki 1931	rabbits with transplanted tumour (number not stated in English summary)	lead 0.2 mgm.	no effect on the tumour, augments somewhat the glycolysis
Collier 1932	rabbits with Brown-Pearce carcinoma in testicles	lead prep. 'R 232'	certain inhibition in growth of primary tumour, prevent or decrease the metastasis
	2 test and 2 control animals	high dosage	
	5 test and 2 control animals	low dosage	
Maxwell, Bischoff & Otteri (1931)	rats with transplanted tumour 14	leadortophosphate and leadacetate	no effect
Datnow, Beilensohn, Howard, Heilbron, Jeffcoath & Dilling 1935	rats with Brown-Perace's tumour	diff. lead compounds, 0.5 % lead in solutions	lead-benzenesulphoglycinate gives best effect
Kikuchi 1936	rabbits with Kato's sarcoma, number not stated, but totally 13 groups treated, and 5 non-treated	colloidal lead prep. + ray treatment	effect of the treatment proportional to the given dosage of radiation
	Experimental investigations comprising larger material.		
Mottram 1928	mice with transplanted tumour 22	colloidal lead + radium treatment	tumour disappeared in 8 animals (this is ascribed to secondary radiation from the lead)
Krebs & Clemmesen 1934	mice with transplanted tumour. 3 groups comprising 100 animals, of which 60 test and 30 control animals	lead prep. 'R 232', 'R 237' immediately before and after transplantation respectively, on fully developed tumour	no healing effect at all

Author	Size and type of material	Methods of treatment	Results
Liverpool Medical Research Organisation 1935	rabbits with Brown-Pearce's tumour. Very small sets	27 different lead prep.	except influence on blood pressure, breathing and pulse, no effect
v. Pastinszky & Ottenstein 1935	rats with transplanted tumour 8 groups compr. 8 animals = 64	differ. conc. of colloidal solutions of lead-titanium acid, injections in the tumour	admin. higher doses give some increase of the growth of the tumour, lower doses no effect
Woodhouse 1936	mice with tar tumours appr. 500 in groups of 20	lead pyrophosphate	slight prolongation of the time before onset of tumour from the beginning of painting with tar. Uncertain inhibition of develop. of tumour
		lead carbonate	o positive effect
		lead trypan blue	o effect
		lead trypan red	o positive effect
		lead pyrrol blue	o positive effect
		colloidal lead compounds (stannic, antimony, cadmium, bismuth)	some inhibition of development of tumour from wart stage, but slight difference when comparing with control animals
		lead fluoressein	o effect
		lead nucleotide	o effect
		lead neosalvarsan	o effect
Smaller casuistic information concerning investigations on human beings.			
Martland, Sochokey & Hoffman 1927	patients with cancer 15	colloidal lead prep.	no effect on the tumour cells
Soiland, Costolow & Meland 1927	patients with malign. tumour 12	colloidal lead prep.	2 cases showed clear improvement
Kaemmerer 1928	patients with cancer 14	colloidal lead sulphide	absolutely without effect
Soiland, Costolow & Meland 1928	patients with malign. tumour 18	colloidal lead sulphate	temporary improvement in some cases, in 1 case disease entirely arrested
Barlow 1929	patients with inoperable cancer (number not stated)	Todd's coll. selenium-lead	favourable improvement in som cases.
Feci 1929	patients with malign. tumour 5	Chistonis' coll. lead prep.	1 case (lymph sarcoma) cured during 2 years
Antionioli & Villata 1930	patients with cancer (number not stated)	Dezanis' lead prep.	in 3 cases slight decrease of primary tumour in beginning, in 2 cases slight decrease of lymphatic gland with metastasis
Mattina 1930	patients with inoperable cancer 20	coll. lead	no effect
Wang, Watson & Curran 1930	pat. with malign. tumour 4	leadacetate	no result existing cachexia

Author	Size and type of material	Methods of treatment	Results
Duroux 1931	pat. with cancer (number not stated)	small doses coll. lead; recommends in addition radiation treatment	besides 2 cured cases, often more or less lasting improvment
Collier 1932	pat. with inoperable cancer 20	lead prep. 'R 232'	no reaction in pat. Uncertain effect on tumours
Aub & Smithwick 1933	patients with cancer mammae 12	colloidal lead orthophosphate with simultaneous radiation treatment	uncertain, or negative result
Dalamier & Schwartz 1934	pat. with cancer 17	coll. lead prep. org. lead prep.	only 5 cases followed satisfactorily; in no cases certain effect of treatment
Horwitz 1934	pat. with inoperable mammary cancer 1	coll. leadphosphate	size of tumour decreased and it became movable. Duration of observation not sufficiently long
Gray & Greenfield 1937	pat. with cancer of rectum 1	lead prep.	at section accumulation of lead in tissues established, but in spite of this no inhibitory effect on growth of tumour
Clinical investigations comprising larger material.			
Fitzwilliams 1927	pat. with malign. tumour 60	Bell's prep.	improvement in 2 cases
Stone & Craver 1927	pat. with malign. tumour 21	coll. lead	in 8 cases regressive changes in tumour
Cancer research committee of the General Hospital, Birmingham 1928	pat. with cancer (19 women, 15 men) 34	Bell's coll. lead prep.	9 cases improved, unaffected 8, 7 died of cancer, 3 died of the treatment
Wyard 1928	pat. with malign. tumour 88	coll. leadhydroxide 32 lead treatment acc. to Bell 56	good effect in 1 case, on the whole a difficult and dangerous method
Bell 1929	pat. with inoperable cancer 566	metallic suspension or coll. leadphosphate	2 cases believed cured and died of other diseases, in 12 cases disease entirely arrested in 51 cases cured and treatment finished
Dentici, Moratti & Pattarin 1929	pat. with malign. tumour 25	different coll. lead prep.	1 case seemed clinical. cured, 1 case improved, 16 cases died, 7 cases are still treated
Ullmann 1929	pat. with malign. tumour 57	tetraethyllead	3 cases improved, 2 cases considerably improved, lived more than a year, 5 cases considerably improved, but now dead
Brunton 1930	pat. with inoperable cancer 38	Bell's prep. 'S 7'	in 3 cases noticeable inhibition of growth of tumour, 2 cases clinically cured after 2 years

Author	Size and type of material	Methods of treatment	Results
Knox 1930	pat. with malign tumour 60	lead prep., in addition many were treated with X-rays. Lead treatment acc. to Bell	20 are excluded because they got less than 150 mg. lead, the remaining 40 were advanced cases. In 1928 8 lived, of which 4 were clinically recovered. In 1929 5 lived, 3 were in good health
Moglia 1930	women with genetic cancer 26	lead treatment acc. to Bell	3 cases clinically cured, 2 cases improved
Polichetti 1930	pat. with cancer in different organs 38	coll. lead prep.	no favourable effect on tumour or on patient's general health
Todd 1930, 1934	pat. with malign. tumour 155	coll. lead-selenium compound + radiation treatment	24 seemed recovered after 2 years, 6 died of intercurrent diseases and had no tumour when sectionalizing
Bargen, Bayard & Osterberg 1935	inoperable and severe cases of cancer 81	coll. leadphosphate 400—800 mg. lead	14 cases were free from cancer in 2 years, of these 7 were treated with lead only
Gould, Kullman & Shecket 1937	pat. with inoperable cancer 22	coll. leadtriphosphate	in most cases progressive anaemia
Roffo & Calcagno 1938	pat. with malign. tumour 70	coll. lead	no improvement, in some cases increase of size of tumour

more casuistic in type, and the second comprising more elaborated experiments with almost satisfactory material as regards the size. As is apparent from the tables, the examinations have on an average given negative results. Among the more satisfactory investigations which have yielded negative results, the work of KREBS, CLEMMESEN and WOODHOUSE must be mentioned. On the other hand, MOTTRAM obtained positive results in so far as the tumour disappeared in 8 out of 22 mice with induced tumours, but this may be due to the simultaneous radium treatment.

After BELL considered that he had succeeded in curing some chorion epithelioma, he continued to try the effect of colloidal lead preparations, and also on other types of malignant tumours, since these preparations seemed to possess tumour-affinity. As the driving force of a team of investigators at Liverpool, he published in 1929 a survey of their results. 566 patients suffering from histologically verified, clinically inoperable tumours were treated with colloidal lead preparations. Of these cases 2 patients were considered as cured (see table), and died from another disease $4\,^1/_2$, and $5\,^1/_2$ years later; 51 cases were considered as cured (duration of observation not stated) which is why the treatment was discontinued, and in a further 12 cases the cancerous disease was arrested. The author states that injections with lead preparations were probably

the cause of death in certain cases because of lead poisoning, particularly in those cases which were treated policlinically in the beginning of the examinations during which the patients could not be controlled with sufficient care. I cannot with certainty state whether the patients were actually cured, because I have been unable to find any published post-observations of the same material, and neither has any non-treated control material been found.

As a consequence of BELL's results, many examinations with the same or similar preparations were made. Details appear from the table.

Among the more satisfactory examinations which have given negative results, those of FITZWILLIAMS, POLICHETTI, ROFFO & CALGAGNO, WYARD should be mentioned. On the other hand, ULLMAN, KNOX, MOGLIA, TODD, BARGEN & BAYARD & OSTERBERG have obtained positive results in so far as they consider that they have observed several cases of clinical recovery. TODD employed, apart from colloidal lead-selenium compound, ray treatment also.

When judging the results of clinical examinations, it must be remembered that one occasionally obtains a cured case due to an erroneous diagnosis. Any significant number is of course out of the question. Rare cases of recovery can, however, be due to such a diagnosis. Furthermore, one has not quite clear conceptions as to how long a patient can be almost free from symptoms and improve, especially if he has been subjected to another kind of treatment, for example X-ray. Our knowledge as to the prognosis, and length of life for cancer cases which are not treated is very incomplete. It is obvious that the majority of cases, which are diagnosed during their early stage, receive some treatment; therefore one does not know how the cases would have developed if they had not been treated. A certain variability which is difficult to judge exists in each case. With respect to these sources of error, one must not consider that a positive result with any method of treatment exists, unless the results are more positive in general. Rare cases of improvement must be judged with very great caution.

It is thus very doubtful whether one dares take the risk of ascribing any certain effect to the treatment of malignant tumours with colloidal lead preparations, as the material of cancer cases which have been treated only with lead preparations is too small, and besides this, sufficiently comprehensive control material of non-treated cases is missing.

Those investigations performed with salts of thorium are difficult to interpret inasmuch as the effect can, in part, depend upon the radiation following the metal ion.

As appears in the table, the effect of thorium on tumours was examined at an early stage in the belief that thorium should possess a certain tumour-affinity. It appeared that the tissues of the tumour had no affinity to thorium in salt solutions.

Among experimental investigations on more satisfactory material

2. Thorium.

Author	Size and type of material	Method of treatment	Results
	Experimental investigations with smaller sets.		
Keysser 1913	mice with transplanted tumour (number not stated)	mesothorium	cured in certain cases
Kawata 1929	mice with transplanted tumour — up to 5 in each experiment	1.0 mg thorium B	no direct influence on tumour cells
v. Hevesy & Wagner 1930	mice with transplanted tumour 7 control animals 7	thorium nitrate injections	no selective accumulation of thorium in tumour tissue
Maxwell, Bischoff & Ottery 1931	rats with transplanted tumour 11	thorium chloride intraperitoneal	no effect on tumour growth
Ehrenberg 1932	mice with transplanted tumour, 6 experimental series with 2—5 mice	thorium B injections	tumour absorbs after repeated injections less thorium than other tissues
Montanari-Reggiani 1933	mice with transplanted tumour (no stated number in report)	thorium nitrate subcutaneous every or every 2nd. day	clearly inhibitory influence on tumour growth, in some case regression and complete disappearance of tumour. No accumulation of thorium in tumour tissues could be observed
Prussia 1936	rats, old 7, rats young 7	15 subcut. injections of thorotrast	5 of 6 surviving animals got spindle cell sarcoma which could be transplanted into other animals
Schürch & Uehlinger 1936	rabbit 1	mesothorium placed in thigh	Ewing-sarcoma
Dittmar 1938	mice with transplanted tumour (no stated number in rep. 2?)	thorium-B in coll. solution, injec. intraperitoneal, intravenous and intralumbar	no accumulation of thorium in tumour tissues
Onufrio 1938	rabbit 1	intravenous injec. of thorotrast	2 years later tumour and liver and spleen
Foulds 1939	young female guinea-pigs 20	thorotrast, 4 injec. at the base of nipple	carcinogenic eff. first tumour observed 138 weeks, totally 4 malign. tumours
Miyamoto 1939	rats 13, rats 8	0.5 ml. thorotrast subcut. thorotrast intraperitoneal	sarcoma obtained in 6 cases of which one could be induced in 11 generations

Author	Size and type of material	Method of treatment	Results
	Experimental investigations comprising larger material.		
Spies 1931	mice: males & females with transplanted tumour 8 groups with 10 mice	1 % uranium + 20 % thoriumhydroxide	after termination of exp. 5 test & 5 control animals lived. Of the remainder average length of life for control animals was 32 days, and for test animals 26 days. Absorption of tumour and sometimes cancerous lymph glands through treatment
Oberling & Guérin 1933	rats with transpl. tumour 20	thorotrast injections intraperitoneal	in 10 cases a decrease in size of tumours, in 8 of these intraperitoneal sarcomas.
Roussy, Oberling & Guérin 1934	rats 15	thoriumdioxide intraperitoneal 10 and subcut. 5	after 1—1 $^1/_2$ years 8 animals developed sarcomas, no metastases
Roussy, Oberling & Guérin 1936	rats with transplanted tumour 20	thorotrast intraperitoneal	tumour was re-formed in 10 animals which died one year later of peritoneal sarcoma
	control rats 10	thorotrast intraperitoneal	died prematurely from infection
	rats with transplanted tumour 10	thorotrast subcut.	7 died of tumour, in 3 tumour disappeared
	rats with transplanted tumour 10	thorotrast intraperitoneal	5 died of tumour, in 4 tumour disappeared. Of the 7 surviving animals in the two last groups 2 developed sarcoma in peritoneum and 1 subcutant
	rats with transplanted tumour 10	thorotrast intraperitoneal	in 9 resorption of tumour, of these 7 developed sarcoma in peritoneum
	rats with transpl. tumour 10	thorotrast intraperitoneal	all died of tumour
	control rats 10	thorotrast intraperitoneal	7 developed sarcoma
	normal mice	thoratrast intraperitoneal	no tumour
	hens	thorotrast intraperitoneal	no tumour
Selbi 1936	rats 60	thorotrast subcut.	14 developed sarcoma
	mice 60	thorotrast subcut.	2 developed sarcoma all after 3 months
Roussy & Guérin 1941	normal rats 16	thorotrast subcut.	11 developed sarcoma
	normals rats 36	thorotrast intraperit.	25 developed sarcoma
Heiman 1942	female rats 30	thorotrast injec. 27	treatment causes no change of benignity of tumour
	with spontaneous mammary tumour or benign ind. tumour (fibroadenoma)	thorotrast + esteroninjec. 9	

Author	Size and type of material	Method of treatment	Results
		Clinical investigations.	
Hoede 1930	malign. skin tumour in patients 5	thorium-X-tubes	healing
Pach 1930	patients with inoperable cancer 8	uranium-thorium in coll. solution	in 1 case of mammary tumour, temporary improvement of skin metastases, in 7 cases no effect
Hoching 1928	patients with inoperable cancer 31	1 per cent uranium + 20 per cent thoriumhydroxide	in 70 per cent the tumour softened

with negative result those of HEIMAN must be mentioned; he found no effect of thorotrast on spontaneous or induced mammary tumours in rats. On the other hand, ROUSSY & OBERLING & GUÉRIN obtained from several investigations a positive result inasmuch as they, it is true, obtained resorption of an induced tumour in many rats, but later on sarcoma arose in several rats. Thus they could, as SELBI, establish a carcinogenic thorotrast effect which should depend on radioactive influence through chronic irritation (compare those of CLUNET & PIERRE MARIE & LAPOINTE with radium induced tumours in rats).

It is evident that patients with malignant skin tumours can be cured through treatment with thorium-X-tubes. As regards attempts to treat malignant tumours with a combination of uranium and thorotrast HOCHING informs that in 70 % of the cases the tumour softens. On the contrary there does not seem to be any lasting inhibition of the development of the tumours.

Certain dyes such as isamine blue, trypan blue, pyrrol blue, and others, posses tumour-affinity i. e. when sufficient quantities are injected they stain, it is true, the whole body except the central nerve system, but when tumours are found it is assumed that most of the dye is accumulated in the tumour which is why this becomes particularly highly coloured. This circumstance has been made use of in tumour therapy, as it has been hoped that those dyes selectively accumulated in the tumour tissues in this manner should, through influence on the enzyme processes of the tumour cells, inhibit the growth of the tumour and eventually prevent its metastases. This capacity to selectively stain the tumours is the object of discussion in the literature, as well as the results of the treatment. The results appear from the table. Among the investigations on more satisfactory material with negative result LIGNAG & KREUTZWENDEDICH v. d. BORNE deserve mention. They even belived they observed a quicker growth of the transplanted tumours in mice which were highly stained with trypan blue. On the other hand, IMAGAWA thought he

B. Treatment of Tumours with Dyes.

Author	Size and type of material	Methods of treatment	Results
		Experimental investigations on smaller sets.	
Baldwin 1923	rats (number not stated)	trypan blue + radium treatment	treatment with trypan blue before gives increased radiation effect
Sugiura & Benedict 1929	rats with transplanted tumour hens with transplanted tumour (number not stated)	congo red gentian violet malachite green methyle blue	somewhat inhibitory influence of malachite green and gentian violet on growth of tumour
Vecchi 1930	rats w. transplanted tumour (number not stated)	trypan blue + radon	in some rats inhibited growth, in others decreased rate of growth
Kreutzwendedich v. d. Borne 1932	mice with tar tumour (number not stated)	isamine blue	inhibited development of tumour. Benign tumours increased, not cancer
Lewis and Lewis 1932	hens with transplanted tumour, rabbits with transplanted tumour	80 different dyes	no certain effect
Brooks 1934	rats and mice with transplanted tumour (number not stated)	orthochlorophenol-indophenol, orthro-cresolindophenol, methylene blue, neutral red, brilliant cresyl blue	methylene blue inhibited somewhat growth of tumour the remaining without effect
Minegishi 1935	rats with transplanted tumour (number not stated)	isamine blue	high dose promotes somewhat growth of tumour, but low dose inhibits slightly
Nakamura 1935	rabbits with transplanted tumour rats with transplanted tumour mice with transplanted tumour (number not stated)	methyle blue	only slight inhibitory influence on growth of tumour
Nyka	transplanted tumour	isamine blue pyrrole red	inhibitory influence on growth of tumour
Schiller 1937	rats treated 7, control animals 3	light green F. S. inj. 1—2 times weakly	carcinoma
		Experimental researches comprising larger material.	
Lignac & Kreutzwendedich v. d. Borne 1928	mice with transplanted tumour treated males 39 treated females 43 control males 32 conrol females 30 animals in 13 litters, of which 9 in 9th generation	trypan blue	the stained mouse developed more quickly growth of tumour

Author	Size and type of material	Methods of treatment	Results
Munck 1928	mice with transplanted tumour up to 12 animals in each exp. group totally 130	trypan blue	vital-staining of animals before, simultaneously with or immediately after induction has a inhibitory influence on growth of tumour
Kimura 1929	rats with transplanted tumour sarcoma 60	isamine blue	no effect
		trypan blue	no effect
		isamine cholin	21/60 completely cured no recidivation, microscopically no tumour cells
		trypan cholin	
	carcinoma 75	»	17/75 cured, recidiv. in 3
	rabbits with sarcoma 30	»	13/30 cured
Imagawa 1930	rabbits more than 40 in 4 groups with transpl. tumour	treatment with trypan blue alone or after milt extirpation comp. with control animals	treatment with trypan blue yields slightly less growth of tumour together with milt extirpation increased growth of tumour, metastasizing and growth
Forssmann 1931	mice with tar tumour 20—50 mice in each experiment	*azo dyes:*	following dyes stained the animals temporary so that they already next day were colourless: acid fuchsin, phloxin, neutral red, echtgelb, Bismarck brown, Nile blue. Lasting staining more than 3 months after the last injection: trypan red, water blue, isamine blue, brilliantdianilblau, all tumours treated with these dyes were colourless. Only nachtgrün stained somewhat the tumour tissues, above all, the lung metastases, light green. None of the dyes had inhibitory or promoting influence on the tumours except chrysoidin which in one exp. on 110 mice developed tumour in 30/45, while control animals had cancer in 27/65 (66 % and 36 % respectively)
		a. basic	
		chrysoidin R	
		Bismarck brown 2 R	
		Janus red (B)	
		Janus brown R.	
		b. acid.	
		echtgelb G. R. canc.	
		gold orange	
		azidolschwarz 5 BA	
		benzidine dyes:	
		Trypan red	
		Triphenylmethan dyes:	
		a. base	
		malachite green	
		b. acid	
		acid fuchsin	
		water blue	
		Seiden blau BT 5 B	
		brilliantdianilblau 6 G	
		isamine blue 6 B	
		light green 2 GN	
		nachtgrün A	
		phtaleine:	
		eosin	
		phloxin o	
		pyronine dyes:	
		pyronine G	
		rhodamine G	
		rhodamine B	
		rhodamine 3 B	
		oxazin: Nile blue sulph.	
		thiazin: methyle blue rectif.	
		azin: neutral red	

Author	Size and type of material	Methods of treatment	Results
Smaller casuistic information concerning investigations on human beings.			
Wehik 1931	patients with inoperable cancer 10	isamine blue + radiation treatment	improvement in 3 cases
Roffo 1932	patients with malign tumour 4	methyle blue	inhibitory influence on growth of tumour
Russ & Scott 1935	patients with malign tumour	trypan blue + radiation treatment	treatment with trypan blue increased sensitiveness to radiation
Hamperl 1939	patients with colon cancer 1	isamine blue	the section revealed that it was a case of chronic spastic colitis
Clinical investigations comprising larger material.			
Bernhardt & Strauch 1928	patients with malign. tumour 33	isamine blue	in some cases considerable inhibition of growth of tumour and improvement of the patients general conditions
Bernhardt 1930	patients with malign. tumour 79	isamine blue + radiation treatment	in relatively high per cent inhibition of growth of tumour
Zadik 1930	patients with inoperable cancer 95	isamine blue in part together with bismuth	in 8 cases no recidivation 1—2 years after treatment

ascertained a certain inhibitory effect on the growth of the tumour when treating a transplanted tumour in rabbits with trypan blue. FORSMAN tried a great many different dyes on tar tumours in mice, and found that nachtgrün stained the tumour tissue, especially the lung metastases. He further established that chrysoidine increased the incidence of tumours in mice from 27/65 to 30/45 in control animals (according to the present author, difference = 25.2 ± 9.3 %).

Summarizing, it can be maintained about these, as of previously mentioned animal experiments, that the series are rather small, and that when utilizing induced tumours one should have considerably larger material, considering the greater variability in the growth of these tumours and their great sensitiveness to outward influence.

It can be further seen from the table that the clinical examinations on larger material are performed on rather small material. The results should probably be considered as negative. In any case, the reports given are too uncertain and vague to consider that definite positive results exist, except perhaps BERNHARDT's whose results probably also depend on the simultaneous radiation treatment.

When judging the clinical researches, HAMPERL's report is of great

interest. A case of spastic colitis, with the diagnosis cancer of the colon, had been treated with isamine blue and considered as cured through this treatment.

As indicated in the introduction, literature on experimental cancer research has become still more extensive. During the last decade, interest has been more and more turned towards the significance of the various hormones on the origin and development of tumours. In the table only those works have been mentioned which embrace the effect of castration on tumours. The most important results of *castration of female animals* are the following:

Transplanted tumours. Where transplanted tumours are concerned the influence of hormones can partially cause a changed frequency of positive results of transplantations, and partially cause a quicker or slower growth. The conceivable results are, in other words, of two types, partly the onset, and partly the secondary growth. Presumably, in this connection the results run parallel. Among investigations with more satisfactory material with negative results those of Graf & Strong deserve mention. Fellner believed he obtained a certain increase in the growth of the tumour. Baatz on the contrary, obtained a smaller tumour incidence when castrating female mice before induction, in the castrated mice than in the control mice (according to the present author, difference = 55.2 ± 12 %). Pribram believed he established a prolonged average length of life in castrated female mice as compared to the control mice, which should indicate a retarded growth of the tumour. Heiman & Krehbiel transplanted a fibroadenoma on female rats and obtained a lower tumour frequency in the castrated animals (according to the present author, calculated difference = 50 ± 7.72 %). By using this tumour, which according to the manner it reacts could be compared to the spontaneous mammary tumours, the castration decreased the susceptabilities for transplanted tumour formation in females, but seemed to increase it in males. Druckrey established that castration of young female mice increased the gonadtropic hormone content of the hypophysis in the fourth month, after which it decreases in the 7th month. During the maximally increased functioning of the hypophysis, a retarding of the growth of transplanted tumours and a decreased incidence of induction took place.

Tar tumours. Kondo thought he found first a somewhat increased, and then a somewhat inhibited growth of tar tumours in rabbits through castration. When considering the very slow growth of a tar tumour in rabbits this result may be considered as negative. On the other hand, Katz found that castrated female mice developed tar tumour less frequently than normal females (the difference according to the present author becomes at least three times its own standard error). These tumours also give metastases less often in castrated animals (here the difference also becomes more than three times its own standard error).

Spontaneously occurring mammary tumours in mice. While Bischoff

C. Influence of Castration on Tumours.

1. Experimental investigations.

Author	Size and type of material	Methods of treatment	Results
Experimental investigations with smaller sets.			
Sweet, Corson, White & Saxon 1913	mice with transplanted tumour; 50 castr. and 75 normal, sex not stated	castration	castration should make the mice more susceptible to transpl. tumour
Maisin, Desmeth & Jacqmin 1926	male mice with tar tumour (more than 11)	castration	number of animal which lived more than 7 months after beginning of treatment was: 12 normal and 11 castrated males. The castrated animals developed more frequently metastasis, the tumours seemed to become malign. quicker than in non castrated
Parodi 1927	mice with tar tumour (sex and number not stated)	castration	no effect
Nakamura 1928	male doves with transplanted tumour (number not stated in English summary)	castration	better growth of tumour in castrated males
Carranza 1929	female rats with transplanted tumour (number not stated)	castration	no certain effect
Jacqmin 1929	mice with tar tumours (number not stated)	castration	in numerous experiments is proved that before or after puberty the malignity and metastasising of the tar tumour is accelerated
Centanni 1930	male mice with transplanted tumour (number not stated)	castration	no certain effect
Mori & Nakamura 1930	rabbits with transplanted tumour (number not stated in English summary)	castration	females: slight inhibition males: slight increase of growth
Maurizio & Debiasi 1933	female mice with tar tumour 15	castration	in the castrated animals an increased number of tumours
Furth & Butterworth 1936	mice with spontan. mammary tumour	general roentgen radiation	decrease of frequency of mammary tumour
Nitta 1936	white rats with transplanted tumour (number not stated) 12 in each group	castration	the transpl. tumour grew somewhat slower in the castrated rats.
Pico 1936	rats with fibroadenoma mammae (transpl. tumour)	castration	inhibitory influence of castration on females, but not on males
Moore & Melchionna 1937	male rats, tumour through injection of dibenzpyrene in prostata 20	castration	first tumour in normal rats after 117 days, in castrated rats after 83 days. Castration 14/20 tumours, in normal 14/18

Author	Size and type of material	Methods of treatment	Results
Tortora 1937	hens (number not stated) transplanted tumour	castration	the castrated hens lived somewhat longer
Champy & Lavedan 1938	cocks 4	partially castrated	regeneration of testicles and after one year seminom or embryom
Mc.Even 1938	control animals 12, 6 castrated	castration	in the castrated 1 tumour, in control animals 0 tumour
Rarei & Gummel 1938	cocks with transplanted tumour 17	castrated 3 $^1/_2$ months earlier	in the different groups 8 and 4 animals survived after 4 and 11 weeks respectively clear inhibition of development of tumour through castration (not possible to treat statistically)
	17	castrated 2 month earlier	
	17	non-treated	
Furukawa 1939	castrated rats (sex and number not stated)	diet on O-amidoazetoluol	in the castrated earlier and more malignant liver cancer
Giuffrida 1939	female and male mice (number not stated)	castration	no influence on origin and development in benzpyrene-tumour
Morikawa 1939	male rats (number not stated)	castration	only slight effect
Spiegel 1939	male guinea-pigs 3	castration shortly after birth	due to castr. adenoma is formed in the suprerenal bodies, which secrete adrenosteron and cause therefore development of genetical organs. Microscopic control shows that the castration is complete
Tunoda 1939	male rats 100	castration (number not stated) diet on buttergelb	in the normal no liver cancer, nor in the castrated
Rosenbohm 1940	mice of one strain 3 different transplanted tumour tested on groups with 7—12 mice in each	castration	almost 100 per cent tumour in the castrated and normal. Growth equal in both
Gardner 1941	female mice 15	castration	13/15 developed tumour in suprenal bodies which cause clear sign on estrogen stimulation
	Experimental investigations comprising larger material.		
Graf 1909	mice, 4 sets with transplanted with sarcoma 150 transplanted with carcinom 50 animals divided into groups 10—15 normal, or castrated male and female mice resp.	castration	no difference between castrated and normal mice after 10 days; after 15 days 100 % cancer in all groups
Rohdenburg, Bullock, Johnston 1911	male mice with transplanted tumour 190 in groups of 38, control 38, castrated 38	castration	tumour in castrated 24/29 = 83 %, in control rats 32/38 = 81 %

Author	Size and type of material	Methods of treatment	Results
Lathrop & Loeb 1916	mice with spont. mammary tumour (55 castrated)	castration	in castrated mice (in 3 groups) 5 tumours = 9 % developed, while control animals had 60—70 %. Besides which the castrated animals had a longer latent period before tumours
Lathrop 1919	female mice with spont. mammary tumour 38	castr. at 3—4 months	no tumour
	108	castr. at 7 months	14 developed tumour (= 13.0 %)
	35	castr. after 7 months	60 % developed tumour. Normal tumour frequency in the employed species was over 50 %
Strong 1922	mice 200 normal + 200 castrated transplanted tum. (2 different)	castration	castration caused no difference in susceptability for transpl. tumour in this species. No difference between sexes
Fellner 1925	mice with transplanted tumour 320, small groups with 5—10 in each exp. castration: in each exp. 5 mices	castration	slight increase in growth of tumour
Murphy & Sturm 1925	male mice 64 transplanted tumour	castrated at 3—4 weeks, transpl. 4, 6 and 8 months after castration	1) 8/22 without tumour aginst 1/20 of control animals. 2) 8/20 and 1/15, respectively 3) 14/22 and 5/20, »
	female mice 34 ind. tumour	castrated when 3—4 weeks old, induced 8 months after	1) 11/20 at 3 weeks against 7/14 at 3 weeks and 2/15 in control animals without tumour
Cori 1927	female mice of varying age from different strains with a frequency of spontan. occurring mammary tumour in non-breeding females varying between 50 and 78.5 per cent. 100 female mice aged 6—7 months	castration	no mammary cancer whereas sarcoma occurred in 3
Murray 1928	female mice with spontan. mammary tumour 210	castration	tumour in normal mice 367/479 = 80 per cent in castrated mice 36/210 = 17 per cent, average length of time before occurrence of tumour was prolonged from 10.5 to 17.5 months
	male mice 211	castration	no tumour
Matsudaira 1931	guinea-pigs, males and females, some of them castrated 57	castration, formation of tumour through introduction of minute stones into gall bladder	castration of partly young, partly old sexually matured animals: in the castrated animals the formation of the tumour was suppressed

Author	Size and type of material	Methods of treatment	Results
Pribram 1931	female mice with transplanted tumour		average length of life:
	96	bilateral castration	average length 39.6 days
	48	unilateral castration	average length 36.0 days
	153	non-treated	average length 30.9 days
Wagner 1932	male mice, 3 sets of 20 test and 20 control animals	castration	in castrated animals somewhat greater number of tumours and quicker growth of tumour
Pribram 1933	male mice with transplanted tumour		16/25 without tumour against 3/19 when castr. unilaterally and 1/19 in control animals. Also decreased rate of growth with 54, 134 and 195 mg, respectively
	25	castr. bilaterally	
	19	castr. unilaterally	
	19	non-treated	
	male mice 40	20 castrated	castration after transplantation without effect on males as well as females
	male mice 40	20 castrated	
Kondo 1934	rabbits 200, females, tar tumour	castration	in the castrated first a somewhat increased then a somewhat decreased growth of tumour
Druckrey 1935 1936 1937	rats with transplanted tumour 200, the majority are females	castration transplantation with tumour at different times	after castration of young females the content of gonadotropic hormone increases in 4th months and decreases again in 7th months. At maximally increased functioning of the hypophysis of the castr. animals there is an inhibition of the tumour growth and decreased yield of the transplantation
Pribram 1935	male mice, 20 test and 20 control animals transplanted in each group	unilaterally castration	some inhibition of tumour frequency and rate of growth
	20 test and 19 control animals		no effect
Furth & Furth 1936	female mice with spontan. mammary tumour 340	roentgen castration	the frequency of mammary tumours decreases from 17.3 % to 9.7 per cent
Heiman & Krehbiel 1936	rats with transplanted tumour fibroadenoma which gives growth frequency on several thousand rats males 33 per cent, females 66 per cent	castration	tumour growth in normal males 33 per cent
			73 males castr. 60 per cent
			normal females 66 per cent
			24 castr. 16 per cent

Author	Size and type of material	Methods of treatment	Results
Katz 1936	female mice with tar tumour, in beginning 100 in each group, when number less than 50 per cent is not stated	castration	inhibitory influence on development and metastasizing of tumour. After 24 weeks 35 per cent of the normal females have tumours, and 18.5 per cent of the castrated, after 28 weaeks 68 per cent and 25 per cent, respectively Metastasis in normal females 12/63 = 19 per cent; in castr. 3.8 per cent.
Murray 1936	mice with spontan. mammary tumour 1000 aged 8—12 months	1) breeding animals 2) separated from males and females 3) castr.	the average cancer tendency in group 3 decreases momentarily after castration. In mice after 30 months the number of directly seized mice is in group 1: 100 per cent group 2: 40 per cent group 3: 0 per cent
Sauerbruch & Knake 1936	rats, 10 test and 10 control animals in 4 groups = 80 In each group the half were males and the other females	40 castrated, all got injec. with embryonic cells, injec. with cholesterine, milk acid, treatment with lapis	in normal animals tumour in 1 case, in the castrated 5 (1 female rat, 4 male rats)
Tavaras & Morais 1937	female rabbits. 50 tar tumours	25 castrated	3 carcinoma in the castr., 5 in the normal rabbits
Baatz 1938	mice with transplanted tumour normal mice 20 castr. before transpl. 23 castr. after transpl. 28	castr. before and after transpl.	tumour in 18/20, 8/23 and 24/28 respectively
Ito 1939	rats 128	castr. 32 control 32 diet on o-amidoazotoluol for 310 days	surviv. castr. 12 surviv. control 15 micr. examination of 7 from each group gave castr. 6/7 hepatoma control 2/7
Nathanson & Salter 1939	mice C 57, transplanted tumour, control males and females 21 of each sex, castr. males and females 27 of each sex	castr.	no certain effect
Stewart 1939	male mice 80 female mice 51 dibenzantracene-tumour	castr. 49 castr. 33	slight, but not remarkable inhibition of origin of tumour during the first period, later on more tumours in the castrated of both sex (low figures)

Author	Size and type of material	Methods of treatment	Results
Woolley, Fekete & Little 1939	female mice of strain with mammary tumour (number not stated)	castr. 24 hours after birth	tumour frequency in normal mice 50.4 per cent, in castr. females 26.8 per cent; latent period before origin of tumour increased from 461 to 522 days
Heiman 1940	male and female rats 160 partly normal and partly castrated, how many of each group not stated. Transplanted tumour one benign. fibroadenoma, males 103, females 57, appr. 8—10 in each group	castr.	tumour in 49 non-treated normal females fibroadenoma in 66 per cent, fibroma in 28 per cent and sarcoma in 14 per cent. Castr. non-treated females 16, 24 and 14 per cent respectively, and castr. non-treated males 54, 28 and 14 per cent respectively
Bischoff, Ingraham & Clarke 1945	mice with spontan. mammary tumour castr. non-treated males 40 normal non-treated males 40 castr. non-treated females 40 normal non-treated females 40	castr.	no effect of castration on development of tumour
Woolley & Little 1945	female mice with spontan. mammary tumour	castr. from 1st to 3rd day after birth	virginal normal females: 26: 0 tumour, castr.: on 13 aged up to 5 months no tumour, in 21 aged 6—12 months 100 per cent tumour of which 11 on both sides

& Ingraham & Clarke could not find any effect of castration on the origin of tumours in female mice, numerous investigations are found where the investigators believe they obtained an inhibitory effect of castration on the origin of tumours. Lathrop & Loeb found, after castration, a decrease of spontaneously occurring mammary cancer as compared to normal females (according to the present author, calculated difference = 63 ± 3.2 %). On the other hand, the frequency of tumours in castrated females was equally low as in the virginal females (according to the present author, calculated difference = 5.5 ± 3.4 %). Murray when examining a 1,000 mice 30 months old, found a 100 per cent spontaneous mammary tumours in breeding animals, 40 per cent in isolated non-preeding females and 0 per cent in castrated females. He believed that the average cancer tendency sunk momentarily after castration. Furth & Furth contrary to the just mentioned investigators, used X-ray castration and here also found a decrease of the tumour frequency (according to the present author, calculated difference = 7.6 ± 2.6 %). Wolley & Fekete & Little established, besides a

decreased tumour frequency, and a prolonged latent period before the occurrence of the tumour in the castrated females.

Among the examinations with more satisfactory material of the effect of castration on the development of tumours in mice with transplanted tumour tissue, that of Bischoff & Ingraham & Clarke (1) must be named. They could not determine any change in the tumour frequency after castration. Murray & Strong on the contrary, obtained fewer induced tumours after castration of male mice than in normal mice (according to the present author, calculated difference = 24.2 ± 7.7 %). Hiraiwa & Kimura, who transplanted fibroadenoma in the mammæ of male rats by which the tumour frequency increased in the castrated male rats (according to the present author, calculated difference = 27 ± 6.2 %). If one assumes that mammary transplanted fibroadenoma reacts similarly as does a spontaneous mammary tumour to hormonal effect (the result of Hiraiwa & Kimura supports this conclusion), the transplanted fibroadenoma had evidently been inhibited through the influence of normal functioning testicles. When these are removed the inhibition disappears and the tumour frequency increases. The effect of castration on males from strains with a tendency towards spontaneously occurring *mammary* tumour has also been examined. Murray did not obtain any tumour in 221 castrated male mice. Nor could Bischoff & Ingraham & Clarke find any effect by means of castration on a greater material.

Summarizing, it can be emphasized that the research work on the effect of castration on transplanted tumours is conflicting in any case not clearly positive. The positive results can partly depend on the fact that the animals, in certain cases, transplanted too soon after castration; for one knows, that even such a slight operation decreases the general resistance of animals, so that the growth of the transplanted tumour is less during the immediate week after the operation. Taking this into account it is doubtful whether after castration during certain investigations the observed inhibitions of the transplanted tumours' development have to be taken to be hereditary, or if the results are caused by the operation as such, or the change in the hormonal balance through castration.

In cases of *tar tumours* castration can cause a reduced tumour frequency and also an inhibited metastasizing in female mice. The majority of examinations on more satisfactory material are carried out on mice with spontaneously occurring mammary tumour. In female mice castration seems to cause a reduction in the occurrence of mammary tumour as compared to normal bred giving females. Moreover, one investigation indicates that tumours occur later in the castrated animals. In males, castration alone has no effect.

In certain strains of mice one can, after castrating the young animals, obtain a pronounced hypertrophy and even adenomatous formation of the suprarenal bodies associated with their right of emigrant, in relation to

2. Mammary cancer in women.

Author	Size and type of material	Methods of treatment	Results
		Smaller casuistic reports.	
Beatson 1896	Patients with mammary cancer 3	operative castr.	some improvement
Michele 1905	patients with mammary cancer 3	operative castr.	some favourable effect
Cahen 1909	patients with mammary cancer 7	operative castr.	1 lived in good health after 5 1/2 years
Foveau de Courmelles 1922 (cit. Smith)	patients with mammary cancer 8	roentgen castr.	considerable improvement
Taylor 1934	patients with mammary cancer	o own cases	in some cases favourable effect
		Clinical investigations on larger material.	
Boyd 1900	patients with mammary cancer 54	operative castr.	in some cases good effect
Thomson 1902	patients with mammary cancer 80	operative castr.	29 cases improved
Lett 1905	patients with mammary cancer 99	operative castr.	in 36.4 per cent favourable effect. Best age for castr. 45—50 years
Ahlbom 1930	patients with mammary cancer 163	roentgen castr. + radiation treatm. of tumour (147 cases)	no effect with castr. alone (16 cases) on growth of tumour or continued length of life for the patient
Pissareva & Deinéka 1936	patients with mammary cancer 31	operative castr. + radiation treatm.	many cases improved. Above all, some inoperable cases become operable through improvement. The metastases are not affected by castr. but need radiation treatment
Herrel 1937	patients with mammary cancer 1 906 patients without mammary cancer 1 011	comparison of the frequency of castrated	in cancer cases castration occurred in 1.5 per cent and in the others 15.4 per cent
Taylor 1939	patients with mammary cancer 50	roentgen castr.	9 cases showed regression of skeleton metastases, 20 cases improved clinically

of the animals. Perhaps, this partially explains the varying results. Such adenomatous in the suprarenal bodies are observed in both male and female mice.

The above reviewed examinations opine that the resistance against tumours should be different in castrated and non-castrated mice, i. e. it should be associated with the influence of sex hormones. From this

viewpoint, it can be appropriate to be reminded of those investigations carried out by AGDUHR concerning the changed power of resistance in mice with activity in the sexual sphere.

When it is a case of judging the effect of castration on mammary cancer, it can firstly be of interest to mention the peculiar difference found vis à vis mammary cancer in married and unmarried women. In Sweden it was NYSTRÖM who first proved that unmarried women under 50 years of age run less risk of developing such cancer. WESTBERG also found a similar difference on smaller material. The latter author gives an elaborated survey of literature in this field, to which I permit myself to refer to. WESTBERG points out the possibility that the significant difference established in this connection is conditioned by the fact, that in married women the periodically more stable condition is produced through gravidity, while in unmarried the hormonal rhythm continues unrestrained. Accordingly, it should be the changes as such which increase the susceptibility to cancer, and not the larger or smaller hormone content.

Among investigations with positive results carried out on more satisfactory material as to the influence of castration on the development of mammary cancer in women, those THOMSON and LETT should be mentioned; they believed that they found a large percentage of improvement. HERREL found, that the frequency of castration before discovery of the tumour in 1,906 cases with mammary cancer was 1.5 per cent against 15.4 per cent in a material comprising 1,011 women without cancer, and with the same age distribution. As it can be assumed that castration, in the majority of the cases, was performed owing to illnesses which would increase the possibilities for the origin of cancer, it is impossible to draw any definite conclusion from these investigations. The other investigations comprise too few cases, and are not treated with respect to later known factors influencing the occurrence of mammary cancer. Besides this, information is missing as to how mammary cancer behaves in a comprehensive material which has received no treatment at all.

In the preceeding table, a compilation of information is given as regards the influence of castration on prostatic cancer. This problem has not been investigated thorouhgly, and the results obtained are given in an exceedingly vague manner. If a subjective improvement is obtained it is difficult to judge to which extent the results might be conditioned by suggestion. At any rate, no examinations which are of a more exact nature have been carried out, therefore the information in the tables hardly permits any definite conclusion to be drawn.

The improvement might naturally depend upon the fact that the hormone production of the testicles ceases. Unter such circumstances, the experiments with sex hormones deserve to be mentioned.

DEAN, WOODWARD and TWOMBLY have treated 31 cases of prostatic cancer with orchiectomy, and 29 with stilbestrol per os. They found in

3. Prostatic cancer and mammary cancer in men.

Author	Size and type of material	Methods of treatment	Results
	Smaller casuistic reports.		
Young 1936	patients with prostatic cancer 2	orchiectomy	no effect
Munger 1941	patients with prostatic cancer 4	roentgen castration	improvement
Randall 1942	patients with prostatic cancer 5	orchiectomy	1 case lived in good health 6 years after operation, but with sign of metastases
	Clinical investigations comprising larger material.		
Dean, Woodard & Twombly 1944	patients with prostatic cancer 100	orchiectomy or treatment with stilbestrol	gradually improvement
Farrow 1944	male patients with mammary cancer 1	orchiectomy	clinical improvement
Huggins 1946	patients with prostatic cancer 21	orchiectomy	5 of 20 lived more than 5 years after operation and 4 of these showed no sign of tumour
Nesbit, Plumb & Arbor 1946	patients with prostatic cancer 795 75 50	 non-treated orchiectomy stilbestrol	prolonged length of life after castration or treatment with stilbestrol as compared to the non-treated cases
Leucutia 1946	patients with mammary cancer 2	orchiectomy	very good improvement. One of the cases seems cured
Mathé & Ardila 1947	patients with prostatic cancer 94	orchiectomy + transurethral operation	some improvement in many cases

both cases that the pains disappeared, an improvement in the general condition of the patients and a certain regression in the skeleton metastases. After 7—8 months a change to the worse took place. As a sign of decreased activity during that period which corresponds well perhaps to a clinical improvement, a decrease was found of certain serum phosphates which otherwise exist in increased amounts in metastasizing in prostatic cancer. Nesbit & Plumb & Arbor have compared 795 cases of prostatic cancer which, on the whole, were not given ray treatment with 75 orchiectomized patients and 50 treated with stilbestrol. These have not been observed for a sufficiently long time to permit any definite conclusions to be drawn. But they believe they obtained a favourable effect using both methods of treatment. Mathé & Ardila treated fewer cases of prostatic cancer with both castration and stilbestrol and considered that they obtained satisfactory results.

It seems indeed to be that by administering substances which have the same effect as women's sex hormones has an advantageous effect on

the course of cancerous diseases. If this is the case, then it hardly seems probable that the extirpation of the male sex hormones and some of the female sex hormones, i. e. castration, should have an advantageous effect, but of course it is not out of the question.

For when it is a question of castration in men, as well as in women, the effect can possibly depend on secondary atrophy of the respective organs. This atrophy should in its turn diminish the rate of growth in cancer. This in itself seems probable, as men seldomly develop mammary cancer, which naturally depends on the fact that the gland tissues in the absence of hormonal stimulation become atrophied. It seems more doubtful whether castration should have an effect on the origin of cancer. There are very uncertain normal figures for that rate of development which exists. Even if one has control material, it must be choosen in exactly the same stage of development so as to be comparable. A difference may naturally be due to the fact that the castrated were in an earlier stage of development, and therefore had a longer time to live than the non-castrated. It is evident that the age factor must also be taken into consideration in this connection. Younger persons have presumably greater powers of resistance than older ones to cancer, and it seems à priori probable that one is not so inclined to operate on old people, or patients who are in an advanced stage. If these viewpoints are correct they imply a tendency towards incorrect impressions, the influence of which is difficult to judge.

As regards the question as to whether castration decreases the risk of cancer in the organs which are under the influence of sex hormones, the mentioned works can hardly be of any guidance. As pointed out above this is naturally conceivable. The decreased risk can either be thought of as being associated with the rhythmical stimulation which is stressed upon by WESTBERG. The problem of course needs to be analysed further.

Even if it is doubtful whether the treatment of malignant tumours with colloidal lead preparation can be said to have any positive effect, the possibility cannot without further experiments be rejected. It should therefore be of certain interest to test the effect of a low toxic lead preparation on a sufficiently comprehensive animal material.

Only a few experiments with thorium have been made as regards its therapeutic effect on malignant tumours. The result of the experimental investigations mainly revealed a certain sarcoma producing effect of subcutaneous, or intraperitoneal injections of thorotrast in rats. As regards thorium's radio-activity and properties as a heavy ione, it would be of interest to test the effect of a soluble radio-active thorium compound on mouse tumours.

The result of the experiments as to the effect of tumouraffin dyes on tumours are contradictory. Nevertheless, it would be of interest

to test their properties in a chemical compound of a tumouraffin dye and heavy metal ione, such as lead or thorium.

During recent years, the interest in the hormonal effect on tumour growth has ever increased. Castration belongs to one of the most common measures, and in female animals particularly. These experiments have mainly been performed on mice with a considerable tendency to develop spontaneously occurring mammary tumours. They show the special conditions in a tumour originated from an organ the function of which is dependent on the sexual glands. But it would however be more interesting to examine the influence of castration on a tumour which is directly under the influence of the hormones of the glands. The results of previous experiments on induced tumours are contradictory and uncertain. On the otherhand, KATZ has, on a satisfactorily comprehensive material, found an inhibition of the origin and metastasizing of the tumours when castrating female mice with tar-cancer. The results are in their details difficult to judge as the material is not in detail sufficiently accounted for. It is, therefore, of interest to examine how castration influences male mice which are afterwards treated with carcinogenic agents and to verify, and eventually complete Katz's results.

CHAPTER II.

Methods.

1. The test animals and their general care.

The ordinary white laboratory mouse is used. Owing to the difficulties during the war to procure mice, the experiments are performed on mice from 3 different strains. The animals used in the series I, III (experiments with lead trypan blue) and IV belong to one strain; the series V, VI and VII (experiments with thorium trypan blue and castration, and influence of thorium trypan blue on normal animals) belonged to another strain; the series VIII (experiments with thorium trypan blue and castration) and series IX (experiments with lead trypan blue on normal animals) to a third strain. Each batch of animals was divided, with respect to sex, from the beginning into different groups intended to be used as test and non-treated control animals. In this way one obtained, as far as it was possible, the same aged test and control animals, and also equal numbers in each group from each batch.

As earlier investigations (VANNFÄLT) have shown that mice weighing less than 10 g. have poor resistance to the toxic influence of painting with coal tar, the animals used were at the beginning of the experiments aged

2 $^1/_2$—3 months, and weighed about 15 g. As a rule they were controlled 2—3 weeks before the experiments, so that it was possible to observe whether they had brought any infection with them which might possibly have some influence on the results. It also appeared, particularly in the first series, that already before the experiments began there was relatively high temporary mortality among the animals. Owing to this, it is natural that the applicable material was decreased more than was expected from the very beginning.

For each animal a special record with notes as to the weight, data as to the time of origin of the tumours, the continued growth of the tumours and the treatment, was kept. As regards the time of the origin of the tumours it was occasionally a little difficult to state this exactly, as there was often a tar crust on the animal from the previous paintings, or sometimes a sore area on the place where it had been painted. However, this time is as a rule stated with an accuracy of a few days, as the animals were controlled each time they were painted, and after the determination of the painting period at least once a week. Later, one could also in the obduction record verify that sometimes a local hyperkeratosis was found which had been considered as the beginning of a tumour.

The mice were kept in cages of iron net. These cages were divided into 4 sections, measuring 30 × 20 × 18 cm, separated by means of sheet walls. The female mice were kept in every second section and the males between. Every second cage contained test animals and the others control animals, so that draught from windows, light and other possible harmful extrinsic factors should not influence the results of the experiments. Pearce & van Allen have examined the influence of light on induced carcinoma in rabbits. The tumours in animals which lived in daylight seemed to have possibly the greatest malignity, while it was less in animals living in darkness and least in those living in artificial light. Vlès & de Coulon & Ugo believe that they found that darkness had an inhibitory influence on tar cancer in white mice. Schorr & Ssobolewa believe they proved that light of different wavelengths has a different influence on tar cancer in white mice. Information is lacking as to whether the light of the wave lengths not required was satisfactorily shaded, and how they produced complete darkness. The material for these experiments generally comprises too few cases to allow any conclusions to be drawn from the results. Seeling & Cooper believed that they more often obtained tar cancer in 125 mice which lived in darkness than in the 125 mice which lived in daylight (according to the present author, the difference = 4.8 ± 3.1 %, and is thus not statistically probable). Thus, the results are conflicting and allow no definite conclusions to be drawn.

The mice were fed daily on white bread and water. Besides this, they were given milk three times a week. The animals in the series VIII and IX were looked after in the same way as were the mice used earlier, but

they were fed on tested food in the form of a special bread containing all necessary ingredients for mouse food, and due to this it was considered that they needed no milk. It appeared, however, that the mice in these series died much earlier than those in the immediately preceeding series; this is equally true of test as well as control animals and without any provable external influence. As these series were infected (epidemic diarrhoea) it is clear, that the animals died sooner.

2. Treatment for the induction of tumours.

The preparation which was used for the production of tumours was coal tar, which under the name of "Saxolintjära" was sold by Fjeldhammers Brug in Norway. This tar has been used for many years by KREYBERG and VANNFÄLT with good results; it consists of a mixture of tar products from many different gas works. It is generally produced from English coal and distilled at 270—300° C., as the preparation is a rather thick liquid. Therefore, it has been heated on a water-bath to approximately body temperature by doing which it becomes more fluid.

When used for painting, the tar is transferred from its large storing container to a glass jar in a quantity of 50—60 ml. If relatively few mice are painted, this amount of tar is sufficient for several paintings. It could be seen how the tar at room temperature gradually became thicker in consistency. However, as the carcinogenic agents in the coal tar could be distilled away firstly at a temperature above 300° C., this little, though repeated heating to body temperature on the water-bath should not cause any evaporation of these agents. On the other hand, it is conceivable that a somewhat increased carcinogenic effect is obtained through a concentration of the tar due to evaporation. An eventual source of error is excluded because both test and control animals were painted each time.

There appeared to be some difficulties in procuring more tar from the same firm as previously during the war. Considering this, and the high mortality in the first two series the present author decided to use synthetically produced carcinogenic substances for the latter part of the examination. According to reports in literature dibenzpyrene and methylcholanthrene are the best. In the future therefore, a 0.3 per cent solution of dibenzpyrene was used, and (when this was not available on the market) a 0.3 per cent methylcholantrene solution, both in benzene. In order to diminish the consumption of this preparation, the mice were first painted with tar for two weeks so as to remove their hair. A drop of the solution was dropped with a glass rod onto the naked skin 6 times a week. In this way the animals were subjected to the most similar treatment possible. The hair as a rule gradually grew, but generally it was not fully grown when the treatment with the carcinogenic agent terminated.

3. Macroscopic examination of the mice.

The chest and abdomen of the dead animals were opened, and then fixed each in their own glass jar in a 10 per cent formalin solution. The formalin in these jars was renewed twice with a week's interval between. When the animals were fixed, an obduction was carefully made. Firstly the tumours, and then the painted portion of the skin were examined. When no tumour was found a preparation was taken from the skin suspected as being the beginning of a tumour formation, for microscopic examination. The complete tumour was carefully removed. The regional lymph nodes were carefully loosened. There are generally four, and one is situated in each axilla, and one immediately behind the shoulder region, or sometimes inserted between the latter and the chest forwards towards the axilla. As a rule they were easy to find. Sometimes it occurred that only three nodes could be found. This could be due to the fact that either one node was missing, or that it was so small that it could not be seen in the tissues. The four nodes could sometimes have a combined weight of 10 mg. Finally, it also occurred that such a small, apparently normal node broke while it was cut away from fat and connective tissue so that it could not be preserved. In those cases where one or more lymph nodes were infiltrated with the tumour tissue, which had grown onto the nodes, the diagnosis metastasizing macroscopically was then given. In the obduction record it was noted, when one node was missing, if it had disappeared, or if it had broken. If it was a case of a very small lymph node, with an otherwise normal appearance, or absence of the tumour in the region gave reason to assume that there was no node, I have considered that no metastasizing existed in the node. These doubtful cases were seldom, and occurred in both control and test animals. The internal organs were extirpated and studied carefully, all lymph nodes and preparations from tumours and macroscopically suspected organs were kept for the production of microscopic preparations. Tumours, lymph nodes and internal organs were weighed. If the weight was under 0.5 g. they were weighed on a torsion-scale with an accuracy of 0.001 g., in other cases an ordinary analysing scale with an accuracy of 0.001 g.

The lymph nodes were examined in all animals with tumours. If it were a case of very large nodes with macroscopically visible metastases, a section of these nodes were cut away for sectionalizing together with the smaller.

4. Microscopic examination.

Some sections from varying depths of the preparation from the tumours and internal organs were taken, while serial sections were taken from the lymph nodes, and every tenth section was laid on the object-

plate. In this manner it was ensured that even very slight metastases in the lymph nodes would be ascertained, and the possibility that some would not be observed was slight. The microscopic preparation was coloured with hematoxylin and picric acid — acid fuchsin according to van Gieson. The sections were then examined carefully.

5. Statistical Methods.

When preparing this investigation general statistical formulae have been used. When calculating the standard deviation, σ, the following formula has been used:

$$\sigma = \pm \sqrt{\frac{\Sigma a}{n}}$$

and when calculating the standard error, $\varepsilon(M)$, for the standard error of the average, the following formula is used:

$$\varepsilon(M) = \frac{\sigma}{\sqrt{n}}.$$

When the observed number is less than 50, a correction has naturally been made with $\frac{n}{n-1}$. These formulae have only been given in order to avoid misunderstanding as to how the standard error is calculated.

CHAPTER III.

Examination of the Tumours, their Development and Metastasizing.

1. The local influence of tar on the skin.

The mice were painted with tar 3 times a week for 20 weeks on a part of the skin, a little smaller than a farthing, between the shoulder blades, series I, III and IV. Series V, VI and VIII firstly received tar-painting during the first two weeks, and were then treated with benzpyrene, and during the latter part of the treatment of series VI, and during the

whole period of series VIII methylcholanthrene instead, for test and control animals similarly.

At the first painting the hair began to mat together. The animals scratched themselves, and eachother, and bit the tarred skin in order to get rid of the tar. Sometimes one could see them rubbing themselves against the walls of the cages. Some of the hair then partly began to loosen mechanically through which a certain skin irritation appeared. This became more evident as the moulting increased during the first weeks. Already after the first weeks the moulting may be considerable. After 2—3 weeks there was a bald patch of at least 1 cm^2, in size on the back between the shoulder blades. This patch was often pale red surrounded by normal pale skin. It seemed, however, that generally it was not infiltrated. Only in the smaller mice and in those toxically sensitive to the tar, could one as early as this observe a certain scurvy of the skin. The majority of the mice had already after some weeks pale, smooth, bald skin which was not irritated before the tar-painting, but it could become slightly red and irritated the following day after painting. The local changes in the skin after tar-painting has been studied in greater detail by KREYBERG. He has proved that the skin retains its redness up to 48 hours after the first tar-painting. He has shown further that the tar attacks the contractile elements in the walls of the capillaries of the skin, which causes a development of telangioma. There is a tendency towards hyperemia and transudation into the tissues, which during the first months is supposed to be reversible, but later stable.

The local moulting is stated as being different at different examinations, probably because different preparations were used (LIPSCHÜTZ, JORDAN, DÖDERLEIN). Similarly to VANNFÄLT, I have found that the local moulting does not as a rule begin before a week after the commencement of tar-painting. Later, greater variations arise. Some mice remain bald some weeks after the dermination of the painting. Others could re-grow short sparse hair after some weeks. MERTENS believes that hair regeneration is due to strong vitality in the animal and that inability to regeneration is associated with exhaustion. VANNFÄLT observed in glycose-treated and control animals an equal extension of alopecia, though it appeared that glycose had an inhibitory effect on the tumours' metastasizing. According to my own experiences an extensive, or possibly a subtotal alopecia indicates that the animals are in a poor condition. The majority of these almost completely bald animals died shortly afterwards. Those animals that resisted well the tar-painting increased in weight inspite of it, and had besides this strong growth of hair on the painted area. It is also palpable that together with the good condition of the animals there followed a considerable better quality of the fur. It kept cleaner and more shiny. Similarly to MERTENS and VANNFÄLT, I also have the conception that a good, eventually repeated, hair regeneration indicates a good general condition of the test animals.

2. Formation of warts.

Schär's investigations as to the influence of the size of the painted area are interesting. When painting, he used a stencil-plate with an area of 9, 18, 36, 72, 144, and 288 mm². The papillomas began to appear after 10 weeks' treatment and their occurrence was evidently independent of the size of the painted area. On the other hand, the malignity arose earlier in animals which had been painted on a larger area. This is quite natural because it is now known that a certain amount of carcinogenic substance is required to produce a tumour. The animals painted in this manner received more carcinogenic substance on the skin the larger the area, provided that the amount of carcinogenic substance per unit area was not altered.

In the present investigation, test and control animals have, as far as possible, been treated similarly. Thus any eventual difference in the incidence of tumours is not because the animals received varying amounts of carcinogenic substances but due to other factors.

Many of the mice in series I and III died early, partly due to the toxic influence of the tar on a sensitive strain, and probably also because this strain was infected. Owing to this, a low incidence of tumours was obtained. Several troublesome epidemics of diarrhoea (probably streptococcous enteritis) occurred during this period of the experiments. During the following series there was a high tumour incidence and this was, at least in part, ascribed to the synthetic carcinogenic agents. Series IV had a relatively low mortality, but on the other hand late occurring tumours. Now, it happened that during series V a promised delivery of dibenzpyrene was delayed, and one was therefore forced to treat the mice in the latter part of the series with painting only.

That period which elapsed before the tumours developed in the 76 female mice painted with tar only was 125.0 ± 3.2 days, as compared to 89.5 ± 2.8 days in the 44 females painted with benzpyrene. Thus it takes longer time to produce tumours with tar-painting only in female mice; so the difference between tar and dibenzpyrene painted female becomes 35.5 ± 4.4 days, and is thus statistically significant.

All the 44 female mice painted with dibenzpyrene from the beginning developed tumours, while 5 out of 81 tar-painted female mice died without having developed tumours. The difference in the mortatility of animals without tumours becomes, when comparing between tar-painted and dibenzpyrene painted mice, 6.3 ± 2.7 % and is thus not statistically probable.

The tumour-age was 135.9 ± 6.6 days for the tar-painted, and 122.5 ± 5.9 days for the dibenzpyrene-painted; the difference becomes 13.4 ± 8.8 days, and thus only a statistically numerical difference is found.

The tendency to metastases appears from the below table.

Metastases	Observed number of dibenzpyrene-painted females with metastases	Expected number of dibenzpyrene-painted females with metastases	Difference
Occurrence of at least, one metastasis independent of its site.	28	39.63	11.63 ± 3.01
Metastasis to the regional lymphatic glands, independent of eventual simultaneously occurring metastasis at another site	23	32.63	9.60 ± 3.85
Metastasis at two or more sites	14	20.70	6.70 ± 5.18

It can be seen from this table that when using tar, metastases are more often obtained in female mice than with 0.3 per cent dibenzpyrene in benzene, as the tumours in a numerically longer time, it is true, give more often metastases, so that the difference becomes statistically significant.

As all the animals in the series belonged to the same strain, it is possible to make a comparison of the results when inducing cancer with coal tar and a 0.3 per cent dibenzpyrene solution in benzol.

It is obvious that this difference between the results of treatment with coal tar and dibenzpyrene respectively, is only true of those doses given during this experiment. If one had continued the treatment with dibenzpyrene and with stronger solutions for a longer time, then the tumours would probably have become malignant more quickly. It is, however, important to bear in mind this difference, when treating the material and studying the results, which the treatment with thoriumtrypan blue and castration, respectively, may have caused on the origin and development of the tumours.

Although sometimes there could be seen a certain roughness and eventual chapping of the skin in a few small mice, which was a further sign of their poor general condition, then, however, certain changes began to take place in the skin after some weeks, or later, antecedent to the formation of tumours. These were a certain local induration of the skin together with hyperkeratosis, and sometimes a slight tendency to chapping with a moist surface. Under microscopic examination, this is seen in the skin as a hyperkeratosis with some increased flaking of parts of the horny layer. Furthermore acanthosis was observed, certain changes in the stratum germinativum but without malignancy in the manner of the cells' growth, oedema in the cutis and a more or less pronounced scattering of the inflamed cells, mainly round cells. During the tar-painting, there gradually appeared on this patch, one or even more small skin warts. In the beginning it was sometimes difficult to differentiate them from small infiltrated wrinkles, but after some days to a few weeks there formed well limited warts, generally small and round in shape, and

the size of a pin-head. In exceptional cases a wart could be seen which had separated itself from its surroundings in 24 hours, where the previous day nothing was visible. In the report of the experiment, the day of the tumour's occurrence is stated as the first day when it could be said with certainty that a wart existed. In my material, the first wart appeared after 6 weeks (7 warts), and the last 57 weeks after the commencement of painting. Definite disappearance of warts, observed for several weeks, were perceived in three cases. One has reason to assume that in these cases it has been a question of benign changes in the skin. VANNFÄLT has also observed a case in which the wart disappeared. During the course of the investigation the tumour's size was registered at each weighing. The tumours generally became the size of a hazelnut, but could often be of an impressive size (photogr. No. 5, 6). The largest weighed 34 g. while the mouse itself weighed 21 g. after the tumour had been dissected.

3. The pathological-anatomical diagnosis of the tumours.

The opinions as regards those properties a tar tumour must have in order to be considered as malignant or benignant are varying. Some investigators consider that a tumour must have given metastases in order to be considered as malignant. DÖDERLEIN requires malignant degeneration, destructive infiltration in the muscles of the skin, and independent growth after the termination of tar-painting. BORST considers that in many cases, through a morphologic study of the total picture a definite conception is obtained of the malignity of the tumour without positive destructive infiltration into the surroundings being found. DEELMAN mentions precancer, a condition which may develop into cancer. TEUTSCHLÄNDER objects to the conception 'beginning cancer' in such cases where the border between epithelia and connective tissue is intact, even though the appearance of the cells is otherwise typical of malignity. LIPSCHÜTZ has shown by means of transplantation experiments that malignity may occur earlier than can be proved from a purely morphological viewpoint; he implanted warts that showed no histological sign of malignity in the new mice. In one of these mice a new wart developed in 20 days, this soon became malignant. MURRAY & WOGLOM made similar succesful transplantations with tar papilloma and obtained metastasizing tumours in the new mice. According to MOTTRAM the tumours seemed to be malignant or benignant from the very beginning, even though one could not distinguish clinically or histologically between them.

In my experiments, the diagnosis 'malignant tumour' was made if an atypical growing epithelia with a clear diffusion of the border existed between epithelia and connective tissue and beginning infiltration of the

later. Infiltrated growth into the skin muscles or metastasizing has not, however, been claimed. Sometimes it was of course difficult to determine with certainty whether a tumour is malignant or benignant. Such doubtful cases were, however, unusual, and even though one has made new microscopic preparations from these for examination some rare erroneous diagnoses cannot be excluded. The above-mentioned experiments by Murray & Woglom as well as Lipschütz indicate that the border between malignant and benignant should not be made too sharply. The morphologically established malignity is a result of the changed biological properties of the tissues and will appear later. As the material was treated similarly both in the case of control and test animals, that source of error caused by the fact that, in doubtful cases, a benignant tumour was considered as malignant, or the contrary, should be of no importance, because it can only be a question of some few cases. Such a little alteration as to the type of the material causes no noteworthy change in the results obtained from the statistical treatment. However, it is only exceptionally that the diagnoses were given according to the above-mentioned minimum requirements. In general, a more or less marked growth of the tumour into the underlying tissue is found; and besides this in the majority of the cases an already macroscopically visible dispersion to the regional lymph nodes and often also to internal organs.

In the majority of the cases it is a question of epithelial plate cancer with a more or less marked formation of horn pearls. Naturally they vary greatly in appearance, from very small epithelial islands in the connective tissue, which in the absence of horn pearls resemble somewhat a large scirrhus, in other cases large islands of epithelia scattered in the tissue. Sometimes numerous small horn pearls are formed; sometimes large united horn masses are formed, often to a large extent necrotic. Occasionally, practically all the tumours were necroticised so that only with difficulty living parts of the tumour-tissue could be seen in the preparation, even if small sections had been cut from different parts of the tumour. In this case, the diagnosis can nevertheless be easily made because of the occurrence of infiltrating necrotic horn masses.

Often more or less pronounced sarcoma-like tissue can be seen in the tumours. Sometimes they are all spindle cell sarcoma, but sometimes they may be a cancer simplex or eventually cancroid in some of the tumour, while in the rest they look like spindle cell sarcoma. It occurs that the mother-tumour and the metastasis have a different morphological structure, so that the tumour is cancroid, while the lymph node metastases are sacroma-like, sometimes the antithesis occurs. As serial sections of the tumours were not cut, it should be so that both sarcoma and carcinoma tissue exists in the tumour, but that the preparation taken from the microscopic examination only contained by chance one or the other constituent. The azan-staining method of the connective tissue seems to show sarcoma-like cancer in these cases. The tumours

with spindle cell sarcoma in tumour and metastases are regarded as sarcoma. The same irritation of the skin by painting with carcinogenic substances when treating epithelia can cause cancer, while by irritating the connective tissue sarcoma can be caused (e. g. through scratches in the skin). Finally, the same irritation can cause a tumour which has in its different parts a varying structure. In other words, cancer and sarcoma are, on the whole, the result of the same irritation. This is not only true of mice. Even when treating rabbits with carcinogenic substances a tumour can develop, which in one part appears to be cancer, while in the other sarcoma (BURSELL). It is a matter of discussion whether tar-painting can cause sarcoma in mice. KREYBERG and others consider it impossible. ROUSSY, LEROUX and PEYRE maintain that sarcoma is of epithelial origin, and name it "épithéliomes fusocellulaires". But other research workers still, however, speak about true sarcoma caused by tar-painting mice (BANG, BIERICH, DEELMAN, FIBIGER, LIPSCHÜTZ, MOELLER and TSUTSUI etc.). BIERICH & MOELLER transplanted parts of tar tumours, and in favourable cases obtained in this way a growth of either cancer or sarcoma alone. Many mice strains possess resistance against transplanted sarcoma, while others are sensitive, which fact was already proved in 1907 by HAALAND. He induced a mixture of EHRLICH's sarcoma and JENSEN's carcinoma on two different mice strains and obtained a sarcoma growth in one strain, and carcinoma in the other.

4. Microscopical examination of the lymph nodes.

In cancer cases it has been shown that sometimes a very rapid distribution of the tumour cells into the lymph nodes must take place. However, it is some time before any of these cells cause real metastasis. In practice it is necessary to draw a definite dividing-line, even though such a line from a theoretical viewpoint is rather vague. If no cancer cells are found, it is stated that no metastases exist. This judgment is naturally correct in many cases, but may be incorrect in others. As the lymph nodes are destroyed by the examination, it cannot be verified how often this judgment is correct or not. These viewpoints are stated in order to emphasize the relativity of the diagnosis 'free from metastases'. Under such conditions the main thing is that the examination is always made with equal care and the diagnosis similarly.

When studying the material of this examination we have endevoured, as far as possible, to find all existing metastases by means of serial sections of the lymph nodes. In rare cases it occurred that metastases could be observed even though they were so small that they could only be seen on one or two of the observed sections of the gland. The metastases generally had the same appearance as the primary tumour. Horny

epithelial plate cancer was therefore the most common. A mixture of sarcoma and carcinoma was not unfrequently found. The only occurring type of sarcoma was spindle cell sarcoma. The non-specific reaction in the lymph nodes after treatment with tar-painting is an inflammatory reaction which arises as a result of an ulcerated, cracked skin or ulcerated tumour. This results in a swelling of the lymph nodes. VANNFÄLT believed that he found a swelling of such lymph nodes, that were not the site of metastasizing, which became more and more pronounced in proportion to the duration of the experiments. This can, of course, in part depend on the increasing growth of animal, but particularly on the fact that as the animal grew older, an increased swelling of the tumour with increased tendency to necrosis and ulceration and infection took place. Hyperplasia of the lymphoid tissues with sinus catarrh is often seen in the microscope, and naturally mostly in the larger lymph nodes. Necrotized tumour tissue is common even in the lymph nodes. This is particularly true of large glands that may weigh up to, and more than 1 gram. There often occurs a disintegration of the tumour tissue so that it lies within the lymph node capsules, as if in a thin shell. In the section the capsule of connective tissue is then seen on the inner side lined with a narrow border of living tumour cells changing inwardly to dead ones. Infection can also naturally cause abscess and disintegration in the lymph nodes, so that only a connective tissue capsule is seen lined on the inner side with the scattered remains of the lymph tissue.

As appears from the records of the experiments, metastases were observed also in other lymph nodes than the regional. At the obduction, preparations from all macroscopically suspected organs were retained, and naturally enlarged lymph nodes were also included. Lymph nodes suspected as metastases have been observed in the parotic regions, and submandibularly, retro-auricularly, in the mediastinum, in the mesentery and at the inguens. The distribution of metastases in these lymph nodes (apart from the regional) is as follows: 12 neck lymph nodes, 5 retro-auricular lymph nodes, 5 mediastinal lymph nodes, 1 mesenteric lymph node and 1 inguinal lymph node.

Metastases generally take place firstly in the regional lymph nodes. Even though they may occur in other lymph nodes, as appears from the figures above, then it is only in two cases (1 retro-auricular and 1 inguinal lymph node) as they are primary metastases. These two cases need not only depend on a variation of the site of the tumour, but also on the fact that cancerous cells may pass the regional lymph nodes or be distributed by the blood.

These metastases occur in all cases, except two, together with simultaneous metastases in the regional lymph nodes. It is therefore not known whether they might have occurred at primary and more energetic distribution of metastases from the tumour, or whether they are secondary distribution phenomena. It is rather remarkable that only in one case a

primary metastasis has been found in the inguinal lymph nodes. These were examined in those cases where a very large tumour existed which had grown down over the back to the sacral regions. These lymph nodes are normally very small and so were examined microscopically only when they were enlarged. In mediastinum, metastases have been observed in connection with as well as without lung metastases. In all these cases metastases were also established in the regional lymph nodes, which is why mediastinal metastases probably are secondary. Only one metastasis existed in one mesenteric lymph node. This occurred together with metastases in the milt, liver and regional lymph nodes, which is why it must be considered as belonging to a secondary distribution in the blood vessels from the tumour.

5. Microscopical examination of internal organs.

a. *Lungs.*

In white mice a spontaneously occurring, benignant tumour of typical appearance, lung adenoma, often occurs. This phenomena was firstly described by LIVINGOOD (1896) and has since then been further examined by TYZZER and JOBLING. Adenomae are as a rule small, and in rare cases they can become as large as a grain of rice. Jobling once observed an adenoma that was 1.5 cm in cross section. These tumours are well defined and consists of sinuous canals lined with a one-layer epithelia which is as a rule cubic. Necroses are sometimes observed in the centre of larger adenoma. MURPHY and STURM have examined the influence of tar-painting of skin on the incidence of adenoma. In 70 per cent of the mice painted on different places on the skin, and which afterwards lived 1—6 months after the termination, of the painting lung adenoma was found while it did not occur in the control animals. These authors state that as a rule many lung adenomae are found in the same animal; in one case a total of 15 in both lungs. They had sometimes even observed mitoses. They believed that tar-painting causes a "re-tuning" in the animal so that adenomae occur spontaneously. Furthermore, they cannot be formed by the influence of tar-particles which are transported to the lungs via the lymph vessels. The authors emphasize that it cannot be caused by inhalation of tar-particles, but they do not discuss the possibility as regards the inhalation of tar-vapours. GEORGEIEVSKAJA and SCHABAD thought they found that lung adenoma occurred more often in mice whose parents had been tar-painted than in those which were the off-springs of non-treated mice strains. The information in the report does not allow a judgment to be formed from the results of the experiments. CAMPBELL let white mice live in air containing tar-vapours. After about 200 days, skin warts began to form, some of which developed into cancer. In the mice living in tar-vapours, lung adenoma occurred 10 times as often as in the control animals.

In my material two adenomae in the same animal occurred only exceptionally. No mitoses could be observed. The microscopical picture corresponds entirely with previous descriptions. Whether tar-painting is of any significance is difficult to determine.

When judging the figures in the table below, the selection factor must be taken into consideration, that is, in this case the cause of death. If the animals die of cancer, it means that cancer develops less quickly provided they die at a high age. On the other hand, the animals that die early were in other words more 'sensitive' to cancer. It is possible that susceptibility to adenoma in some way is connected with susceptibility to cancer, and that therefore those which die of cancer have a deviating susceptibility to cancer. If the animals die spontaneously of intercurrent diseases, the figures will probably give a more correct impression of the total probability of the development of lung adenoma. If a division of the material is made with this in view, then 3 groups are obtained. Group 1 consists of animals without metastases, and which therefore could be considered as having died of intercurrent diseases (different infections, parasites, intoxication of tar-vapours etc.). Group 2 comprises animals having metastases on one or more places, and have therefore probably died due to the influence of cancer or metastases. Group 3 comprises dead animals from a selection of relatively healthy animals.

Through a division of the material into different age groups, one has not succeeded in finding any clear change with age as regards the possibility of developing lung adenoma. Therefore the table is divided only into two age groups, namely over and under 90 days. It appears, however, as if lung adenoma occurs more often in series I—IV than in the other series, and most often in those that die of intercurrent diseases. It is probable that mice with metastases may have died of intercurrent diseases, which fact contributes to make the dividing line between the different age groups vague. For practical reasons it is not possible to use another dividing line than the occurrence of metastases; this comprises only 18 animals in this series. If groups 1 and 3 in the series I—IV are compared to group 2, lung adenoma is found more frequently in the former than in the latter, the difference is 14.2 ± 4.5 %, and this is significant. In the series V and VI no difference is found, this is probably due to the low adenoma frequency. The fact that adenoma occurs more often in those that die of intercurrent diseases possibly indicates that adenoma carries with it a susceptability to other diseases, especially pneumonia, which cause death.

In the whole material, lung adenoma is found in series I—IV in 12.6 per cent of the cases, and in the series V and VI in 1.5 per cent; the difference is 11.1 ± 2.1 per cent, and is thus statistically significant. No adenoma is found in animals with tumours in series VIII. The difference between series I—IV compared to the other series indicated that *susceptability to adenoma is conditioned by heredity.*

Table 1. *Table of the incidence of lung adenoma at varying tumour-age.*

			− 90 days	> 90 days	Total
Series I—IV	Group 1 death due to disease	observed number	74	30	104
		number of adenoma	8	11	19
		per cent adenoma	10.8	36.7	18.3
	Group 2 died of cancer	observed number	65	75	140
		number of adenoma	4	6	10
		per cent adenoma	6.2	8.0	7.1
	Group 3 killed	observed number	4	14	18
		number of adenoma	1	3	4
		per cent adenoma	—	21.4	22.2
Series V, VI	Group 1 death due to disease	observed number	34	51	85
		number of adenoma	2	—	2
		per cent adenoma	5.9	—	2.4
	Group 2 died of cancer	observed number	40	187	227
		number of adenoma	—	2	2
		per cent adenoma	—	1.1	0.9
	Group 3 killed	observed number	1	76	77
		number of adenoma	—	2	2
		per cent adenoma	—	2.6	2.6
Series VIII	Group 1 death due to disease	observed number	75	24	99
		number of adenoma	—	—	—
		per cent adenoma	—	—	—
	Group 2 died of cancer	observed number	10	6	16
		number of adenoma	—	—	—
		per cent adenoma	—	—	—
Series I—IV, V, VI	Group 1 death due to disease	observed number	108	81	189
		number of adenoma	10	11	21
		per cent adenoma	9.3	13.6	11.1
	Group 2 died of cancer	observed number	105	262	367
		number of adenoma	4	8	12
		per cent adenoma	3.8	3.1	3.3
	Group 3	observed number	5	90	95
		number of adenoma	1	5	6
		per cent adenoma	—	5.6	6.3
	All animals in series I—IV, V, VI	observed number	218	433	651
		number of adenoma	15	24	39
		per cent adenoma	6.9	5.5	6.0

When comparing between the animals in series I—IV with tumour-age under 90 days and over 90 days, lung adenoma is found in 9.1 per cent and 16.8 per cent respectively; the difference is 7.7 + 4.2 per cent and

thus not statistically significant. On the other hand, if only animals belonging to group 1 and 3 are compared with each other, lung adenoma in the same age groups is found in 11.5 per cent and 31.8 per cent respectively; the difference is 20.3 ± 7.1 per cent and thus statistically probable. Age seems to be of some influence on the origin of adenoma, but possibly only to that extent the animals become old enough to develop adenoma.

Košir has painted 150 mice with tar, out of which 63 developed skin cancer. In 7 cases metastasis formed on the lungs. The definite localization to the vessels was remarkable, and he termed it "angiotaxis". This "angiotaxis" it seemed to him was characteristic of lung metastases, and failed to appear in the lymph nodes, heart, kidneys and the milt. Other authors' assertion to the effect that primary lung tumours may develop in tar-painted animals is questioned by Košir. According to Kost three possibilities are found in cases with cancer embolia in the lungs: 1) the cells are transferred to the lungs without giving any reaction to the surroundings and die. 2) the cells may be arrested in the vessels and cause the formation of thrombi. They may either die or survive without further growth. 3) formation of real metastases through the fact that the cells grow through the walls of the vessel, or through continued growth in the vessel. According to Seeman the relative frequency of lung metastasis in man is due to the influence of physical-clinical factors. Seeman opines that the intravascular cytolysis in lungs is a highly specific property of the lungs. Wibeau has examined the incidence of lung tumours in rats and mice treated with intravenous injections of finely divided silt of tumour cells. Some of the animals were instead injected with ascites from animals with intraperitoneal tumour. The silt, as well as the ascites injections, produced tumour in the lungs but not in other organs. This result may be because the silt was too coarse grained so that the cells could not pass the lungs. Contrary to this, Kallos & Kallos-Deffner obtained through intravenous injections of tumour-silt on female rabbits metastases in the kidneys, ovaries, mammae, thyroid and eyes, but not in the lungs, liver and milt.

Fibiger and Möller have endeavoured to bring about metastasizing of tar cancer in white mice in the lungs through treatment with subcutaneous injections of a silt of the skin from the embryo of mice. They suspected that the antibodies which are produced by the embryonic tissue should affect the cancer cells. This treatment did not influence the primary tumour, but decreased by 50 per cent the metastasizing in the lungs and equally as much in the lymph nodes. Waterman tested the influence of freshly made extraction of milt on tar cancer in white mice. Daily, 33 mice received an injection corresponding to 2 gram milt, while 34 control mice received a physiologic salt solution. In the control mice metastases took place in 6 cases, 5 in the lymph nodes and 4 in the lungs. In the mice that were treated, 2 cases of metastases in the lymph nodes were found (the difference as to incidence of metastases, regardless

of localization is, 12.3 ± 7.8; the difference as to lung metastases is 12.1 ± 5.7; the differences are thus not demonstrated). It is possible that cytolytic effect of the extraction, or immunity factors of other kinds, influences the metastasizing to the lungs, but otherwise not. WATSON and MELLANBY presumed they could establish that the number of lung tumours increased if the food was rich in fats. They also state that lung tumours are easier to find in fixed than by fresh animals. VANNFÄLT too has made this observation.

When metastases in the lungs are found, it is probable that the occurrence of such a metastasis is a subsiduary cause of death, if it is not the primary.

Table 2. *Lung metastases incidence in spontaneous dead and killed mice respectively, at different tumour-ages.*

Tumour-age in days			31—90	91—120	121—150	> 150	Total
Series I—IV	Dead	Obs.*	64	39	30	6	139
		Numb.*	12	10	10	5	37
		%*	18.8	25.6	33.3	(83.3)	26.6
	Killed	Obs.	4	—	7	7	18
		Numb.	—	—	2	2	4
		%	—	—	(28.6)	(28.6)	22.2
Series V, VI	Dead	Obs.	40	66	70	46	222
		Numb.	27	46	50	32	155
		%	67.5	69.7	71.4	69.6	69.8
	Killed	Obs.	1	1	10	65	77
		Numb.	—	—	—	21	21
		%	—	—	—	32.3	27.3
Series VIII	Dead	Obs.	10	6	—	—	16
		Numb.	8	2	—	—	10
		%	(80.0)	(33.3)	—	—	62.5

* Obs. = Observed number with malign. tumours. Numb. = Number metastases. % = per cent metastases.

The table includes the complete material which was divided into animals that were killed and those that died spontaneously. As death is more common, the former group is comparatively small, and in series VIII there are none which has been killed.

If one examines the distribution of the number of lung metastases in relation to the number of animals with tumours at different tumour-ages,

an uneven distribution is found with a certain tendency towards an increased number of lung metastases at high ages. As this may be due to different causes of death, the above table has been made. But it can be seen even here, that one cannot advantageously divide the material into equally large groups as there is far too small and uneven a difference between the groups if the complete material of lung metastases is considered.

When comparing series I—IV, and series V—VI one finds in all the age groups considerably higher figures as regards the number of lung metastases in series V and VI, whether or not it is a question of simultaneous metastasizing in other regions. Metastases in the lungs without other simultaneous occurrence of metastases in mice which died of cancer is found in series V and VI to be 15.3 per cent, and in series I—IV 3.6 per cent, the difference becomes 11.7 ± 2.9 and thus statistically significant. Metastases in the lungs with simultaneous occurrence of metastases in other regions is similarly found more often in series V and VI in animals which died of cancer, so that the difference as compared to series I—IV is 31.5 ± 4.9 and is thus statistically significant.

It would, of course, be more correct if when making a comparison between the different mice strains only to use those animals which could be considered as having died of cancer, as the frequency of metastases in a certain organ is compared. It is probable that the frequency of animals which died before they have developed a tumour is not the same, and this condition also makes itself manifest if the obtained number of tumours is compared in relation to the size of the animal material used in the beginning.

In series I—IV, when a comparison is made between the mice with a tumour-age above and below 120 days, lung metastases exist in 41.7 and 21.4 per cent, the difference becomes 20.3 ± 9.2 per cent and thus statistically significant. No such difference can be proved in series V and VI. Thus the tumour-age appears to have a certain influence on the origin of lung metastases, which may become apparent in a strain which has not too strong a susceptability to lung metastases.

In this comparison two additional factors are found which may have some affect on a varying frequency of lung metastases; the one material being painted with coal tar and the other mainly treated with dibenzpyrene. Furthermore, the treatment of the animals may influence the metastasizing. If the incidence of lung metastases is compared in series V and VI between the animals which were only tar-painted and the dibenzpyrene-painted animals which died of tumour, it is found that at a tumour-age exceeding 60 days, 34 out of 50 tar-painted animals have lung metastases i. e. 68.0 per cent, while the corresponding figures for the other animals is 86/169 i. e. 50.9 per cent. The difference between tar-painted and dibenzpyrene-painted animals is 17.1 ± 7.6 and is thus statistically probable. Neither the treatment with lead trypan blue,

thorium trypan blue nor castration has any provable effect on the incidence of lung tumours. In view of what has been pointed out above, *it is probable that the difference between series I—IV and V, VI as to the occurrence of lung tumours in animals that died of cancer depends on a difference in the hereditary susceptability in the strains to develop tumours.*

b. *Liver, milt, kidneys, suprarenal bodies and intestine.*

In this investigation metastases to the lungs were remarkably common, metastasizing to the other internal organs occurred relatively seldom which usually appears to be the case when examining this kind of tumour. The below table gives a survey of metastasizing to internal organs. For the sake of comparison lung metastases are included.

Table 3. *Incidence of metastases in internal organs at different periods from the beginning of painting.*

	Tumour-age in days	31—90	91—120	121—150	>150	Total
Lungs	Series I—IV	18	11	14	7	50
	Series V, VI	27	46	50	53	176
Liver	Series I—IV	1	—	—	—	1
	Series V, VI	1	2	5	1	9
Milt	Series I—IV	1	—	—	3	4
	Series V, VI	1	3	7	9	20
Kidneys	Series I—IV	—	—	2	—	2
	Series V, VI	2	2	4	3	11
Suprarenal bodies	Series I—IV	—	—	—	—	—
	Series V—VI	—	—	2	—	2
Intestine	Series I—IV	—	—	—	—	—
	Series V—VI	—	—	—	1	1

As regards metastasizing to the liver, milt and kidneys also here a certain tendency to a more pronounced metastasizing exists in series V and VI, than in series I—IV. When comparing the size of this difference in animals with a tumour-age exceeding 30 days the following figures are obtained as to the frequency of metastasizing:

Liver: series V, VI 9/382 = 2.3 per cent and series I—IV, 1/223 = 0.4 per cent, the difference is 1.9 ± 1.1 per cent, and thus not proved.

Milt: series V and VI 20/382 = 6.2 per cent and series I—IV, 4/223 = 1.8 per cent, the difference is 4.4 ± 1.5 per cent, and thus statistically probable and almost significant.

Kidneys: series V and VI, 8/382 = 2.1 per cent, and series I—IV, 2/223 = 0.9 per cent, the difference is 1.2 ± 1.0 per cent, and thus it is not provable.

In the liver and milt, metastases are found more often in the animals belonging to series I—IV, but this difference is only statistically probable. When treating animals in series I—III with lead trypan blue an inhibition of the metastasizing to the internal organs was produced, and the difference between non-treated and leadtrypan blue-treated became only statistically probable, thus it is conceivable that this effect can partly explain the fact that the animals in series I—IV seem to have fewer metastases to the liver, milt and kidneys than series V and VI.

I found no metastases to the heart. In some cases widely spread tumours in the lungs were found with distribution in the pleura and even to the external layer of the parietal pericardium, but no in-growth from the mediastinum to the heart.

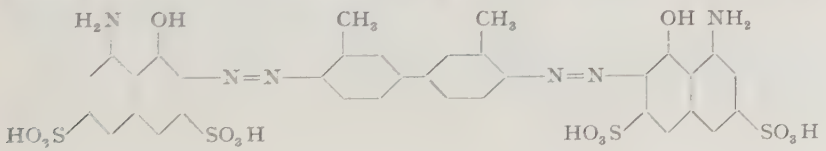
The metastases observed in the suprarenal bodies are not usual. The occurrence of one case with metastasis in the intestine is probably due to a spreading of the tumour to the abdomen, as an abdomenal tumour of similar appearance was found, but no spreading carcinomatosis in the peritoneum. In the same case, a metastasis in one mesenteric lymph node, probably resulting from the intestinal tumour, was also found.

The microscopic picture of the metastases does not in any way differ from those observed in the lungs and lymph nodes. Most often it is a case of cancroid, and in those cases sarcoma metastases are found in the lungs, this also took place in the liver, milt or kidneys.

CHAPTER IV.

Investigation of the Toxic and Pharmacodynamic Properties of Lead Trypan Blue and Thorium Trypan Blue.

For the treatment of tumours it would be advisable to procure a water soluble preparation in which lead and thorium were relatively firmly chemically bound to a tumour affin dye. Such compounds should be only slightly toxic, but should be able to affect the tumours by their accumulation in the tumour tissue. Trypan blue was considered as being one possibly suitable compound for this purpose; its chemical formula is:



The solutions used in the treatment of the test animals have largely been produced according to the following method. The preparation was finely pulverized in a mortar, and then dissolved to saturation in aqua destillata at room temperature. The solutions were prepared every, or every other week. They were stored in a glass flask with a ground stopper, or in glass bottles with rubber stoppers. Before application, it was ensured in each case that the solution was clear. Through evaporation to dryness it was established that the solutions saturated at room temperature contained 10.37 mg. % lead trypan blue, and 3.63 mg. % thorium trypan blue respectively. Through incineration these figures appear to correspond to 4.24 mg. % metallic lead, and 0.87 mg. % metallic thorium. These analyses were carried out according to commonly used methods.

The toxic and biological properties of lead have been known for a long time, and a comprehensive literature has been published. As regards details is referred to Handbuch der experimentellen Pharmakologie Vol. III: 3. It must be mentioned here that mice fail to show symptoms of acute lead poisoning, and the symptoms of chronic poisoning show only a slightly characteristic picture. As one cannot on these animals with certainty find changes in the structure of the blood in the form of fatal chronic blood poisoning, no blood tests have been made on the treated mice. As an example of the toxic affect of lead on mice it must be mentioned that daily subcutaneous injections with lead acetate containing 0.1 mg. Pb has a fatal effect within 1—2 months. A subcutaneous injection of 50 mg. Pb given once and for all in the form of lead carbonate has also a fatal effect within 1—2 months.

Regarding thorium there only exists some little information about its toxic and pharmacodynamic properties. The thoriumdioxide, which was previously often used as X-ray contrast media is rather indifferent. Randerath & Schlesinger state that mice weighing 15 mg. tolerate intravenous injections of 50 mg. thoriumdioxide. Injected with 75 mg. 2 mice out of 6 died.

Contrary to lead, thorium is radioactive. The decomposition of thorium takes the following course: thorium $-\alpha =$ mesothorium; $-\beta =$ radiothorium; $-\alpha =$ thorium X; $-\alpha =$ emanation; $-\alpha =$ thorium A; $-\beta =$ thorium B; $-\alpha =$ thorium C; $-\alpha = ?$

Thoriumnitrate is slightly toxic; when injected subcutaneously it may cause ulcerations. When given intravenously it has absolutely no effect on the circulation, or any other function even if 0.25 mg. per kg. is injected into dogs. Thorium is secreted via the kidneys. The solutions do not affect excitant muscles or hearts from frogs (Chase & Gies, Brown & Sollmann, 1905). The preparation used here is slightly radio active. 1 mg. of dry substance gives 2.02 impulses per minute when determined in a Geiger-Müller-chamber.

1. The Effect of the Preparations on Normal Mice.

During a period of up to 5—6 months, the test animals received such small amounts of lead and thorium (0.83 ± 0.03 mg. metallic lead 0.37 ± 0.01 mg. metallic thorium resp.) that these ought not to have any toxic influence. Due to the slight solubility of the preparations it is not possible to give rabbits a once and for all dose which would injure them when injected intravenously. Hence I have not tested the lethal dose of the preparations. A number of animals otherwise not treated have instead been given the same doses as the cancer animals (subcutaneous injections of the solutions in an amount of 2 per cent of body weight three times weekly), and their length of life etc. was compared to that of the non-treated control animals.

When treating 25 male and 25 female mice which were compared to an equal number of control animals, the following length of life as counted from the beginning of the experiment was obtained:

lead trypan blue-treated males	78.71 ± 4.60 days
lead trypan blue-treated females	92.45 ± 5.27 days
treated males + females	85.62 ± 3.70 days
non-treated males	102.00 ± 5.02 days
non-treated females	78.40 ± 4.76 days
non-treated males + females	90.02 ± 3.80 days

The shortened life of the animals which all died spontaneously is possibly due to diseases (e. g. epidemie diarrhœa) (see page 124). The 50 non-treated animals lived as long as the 50 treated; the difference between non-treated (males and females) and treated (males and females) becomes 4.40 ± 5.31 days. The 25 non-treated males lived longer than the 25 treated; the difference between non-treated and treated becomes 23.29 ± 6.81 days, and is thus statistically significant. Likewise the 25 treated females seemed to live as long as the 25 non-treated; the difference becomes 14.05 ± 7.10 days and is thus not statistically probable.

Corresponding experiments have been made as regards thorium trypan blue. Considering that this preparation was also used in the treatment of castrated animals, its effect was tested on 50 normal and 50 castrated animals; each group comprised 25 males and 25 females. In this series all animals, except 3, survived the experiment and had to be killed.

During an observation period of 6 months, no effect of the treatment as regards length of life could be observed.

Internal organs of the treated animals were examined.

When comparing the microscopical slides from treated and non-treated normal mice no difference between them could be shown. In not stained slides lead and thorium trypan blue can be seen in the interstitial tissue.

2. Effect of Lead Trypan Blue and Thorium Trypan Blue on Surviving Organs.

Adult rabbits were killed by a blow on the back of the neck and slitting of the carotides. The duodenum and the upper third of the small intestine were immediately removed and placed in Tyrode's solution. Approximately 5 cm. pieces of the intestine were mounted according to Magnus' method in the Tyrode's solution in an 80 ml. glass cylinder through which air flowed at a constant temperature of + 38° C. The movements of the intestine were recorded by a kymograph on smoked paper. By means of a pipette the glass cylinders in which the intestines were mounted could be emptied and re-filled immediately. After each examination of the effect of a pharmacon on the intestines, the liquid was changed at least three times with an interval of 8—10 minutes between each change while the kymograph stood still. When the intestine worked normally again the kymograph was started for the next recording. A solution of pharmacon was added by means of a pipette so that the liquid ran down the walls of the cylinder, whereby the mixing with the salt solution was accelerated through the constant air flow.

As only small lead doses were used in the treatment of animals with lead trypan blue, only previous experiments on the effect of small lead doses on surviving intestine will be accounted for here. GRÜNBERG states that in intestine experiments, according to Magnus, with lead chloride, lead nitrate and lead acetate a decrease of tonus is always obtained, and a decrease of the pendular movements in the isolated intestine. In Tyrode and Ringer-Locke-solution a decrease of these movements set in first at 1 : 5 000—1 : 8 000. In a Ringer-Locke-solution without soda already at 1 : 100 000—150 000. These results were obtained when using guinea pigs' intestines. Cholin gives a strong increased tonus in the case of a decreased tonus caused by lead. Furthermore, lead gives a characteristic reaction upon atropinized intestine. An intestine flaccid through lead effect reacts to the addition of barium. Thus the decrease of tonus does not depend either on the paralysis of the vagus, or on a paralysis of the muscles. Then GRÜNBERG continued his experiments with pigeon intestines, and obtained the same effect with lead as with adrenaline. Contrarily, the utherus reaction to lead did not run parallel with adrenaline. He considered that the result indicated clearly that the local effect of lead on the muscles in the intestine and uterus is closely related to the sympathetic innervation and mediated through this.

Own experiments. Lead trypan blue was tested on surviving intestine, a saturated preparation in sterile destilled water being mixed with the double Tyrode's solution. When adding this solution, it was first heated up to the same temperature as the liquid in the glass cylinder. The lead content in the saturated lead trypan blue solution corresponds to the lead content in 6.55 mg. % lead nitrate solution, or approximately 1 :

15 000. When adding 10 ml. of the above mentioned solution in the glass cylinder, it corresponds to a concentration of lead nitrate of 1 : 240 000. If consideration is taken to the fact that the lead, because of its chemical binding to lead trypan blue, possibly possesses a smaller poisoning effect than in lead nitrate, one cannot in view of GRÜNBERG's figures above expect an effect from additions of lead trypan blue in amounts under 20 ml. In view of this, the eventual effects of the additions have a certain interest, as it seems that the effect of such small amounts has not been tried previously on surviving organs.

Addition of 1—5 ml. of lead trypan blue has no effect on the intestine. When adding 10 to 20 ml. lead trypan blue, one has observed in some cases an unmistakable self-effect of manifesting itself in an increased amplitude, which gradually decreases and becomes less than the amplitude before the addition of leadtrypan blue. See figure 1: the amplitude increases within 20 seconds from 15 to 20 mm., while the tonus remains unchanged. During the elapse of 8—9 minutes the amplitude decreases to 15 mm., while the tonus remains unchanged. The frequency did not alter during the whole time. Through adding an equal amount of heated Tyrode's solution, it was made certain that this had absolutely no effect on the activity of the intestine.

Examination of the parasympathetic irritability when influenced by lead trypan blue has given the following results; the addition of 0.05 ml. 0.001 % acetylcholine solution gives a momentary tonus increase of 27 mm., which then decreases within 3—4 minutes to 5 mm. above the starting value. After washing in Tyrode's solution, 20 ml. of lead trypan blue is added, by means of which an increase of amplitude from 15—20 mm. is obtained within 10 seconds with unchanged tonus and frequency. After 15 minutes, when the amplitude is once again 15 mm., an equal amount of acetylcholine is added by means of which a momentary increase of tonus up to + 30 mm. is obtained, and then within 3—4 minutes a decrease to 5 mm. above the starting value. After a re-newed washing, an addition of acetylcholine is made and again a momentary tonus increase up to + 30 mm. is obtained with a decrease within 3—4 minutes down to 5 mm. above the starting value. The addition of 20 ml. leadtrypan blue seems to have no influence on the ability of the intestine-preparation to react on the parasympathetically conditioned motor influence of acetylcholine.

Investigation with adrenaline has shown, that addition of leadtrypan blue stimulates the reaction ability of the intestine preparation to this sympathetic irritant. For small amounts such as 1—5 ml., no positive

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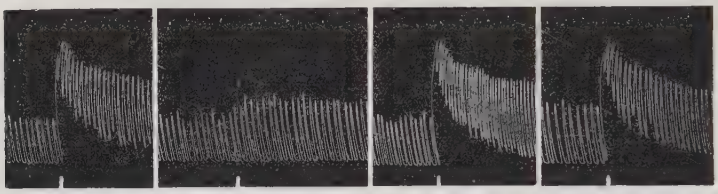
As the curves given here do not included the lower part of the sooted paper ,the time could not be included when cutting out the parts of the curves. When time is stated as "promptly," the typical rapid occurrence of the effect when adding adrenaline, acetylcholine etc. is meant. The dosage of the pharmacon is stated only at the first addition; the concentration is given at the curves.

Fig. 1. Intestine of rabbit.



ac. x l i ac. x ac.

ac. = addition of 0.05 ml. 0.001 % acetylcholine.
x = washing with Tyrode's solution.
l = addition of 20 ml. leadtrypan blue.
i = kymograph immobile for 10 min.

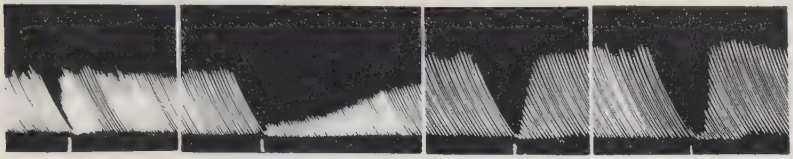
Table 4. *Intestine of rabbit* ($^{26}/_{2}$ *1948*). (Fig. 1.)

Preparation 1. Lead trypan blue + acetylcholine.

Time	Addition	Frequency/min.	Oscillation size in mm.	Tonus in mm.
Before		15	14	—
	0.05 ml. acetylcholine			
Promptly			18—20	+ 27
40″		15	23	+ 8
	Washing with Tyrode's solution.			
Before		15	15	—
	20 ml. lead trypan blue			
After				
20″		15	20	—
15′		15—16	11	—
	acetylcholine			
Promptly			10—15	+ 28
40″		15	21	+ 8
	Washing with Tyrode's solution.			
Before		15	20	—
	acetylcholine			
Promptly			10—15	+ 29
40″		15	22	+ 7

effect is seen. When adding 10 ml. a slight effect is observed, 20 ml. gives a marked effect and this remains, though weakened, even when adding adrenaline after repeated washings. As an example (Fig. 2, Table 5) the following experiment is reported: addition of 0.05 ml. 0.01 % adrenaline

Fig. 2. Intestine of rabbit.



adr. xl. adr. x adr. x adr.

adr. = addition of 0.05 ml. 0.01 % adrenaline.
l = addition of 20 ml. leadtrypan blue + hymograph immobile för 10 min.
x = washing with Tyrode's solution.

Table 5. *Intestine of rabbit* ($^{20}/_{2}$ *1948*). (Fig. 2.)

Preparation 6. Lead trypan blue + adrenaline.

Time	Addition	Frequency/min.	Oscillation size in mm.	Tonus in mm.
Before		16	22	—
	0.05 ml. adrenaline			
Promptly		17	1—2	— 1
After				
40″		17	22	—
	Washing with Tyrode's solution.			
Before		16	23	—
	20 ml. lead trypan blue			
After				
1′		15	24—25	—
15′		15	23	—
	adrenaline			
Promptly		15	2	— 1
After				
40″		15	5	— 1
4′		16	15—16	—
	Washing with Tyrode's solution.			
Before		15	30	—
	adrenaline			
Promptly			1—2	— 1
After				
40″		15	15	— 1
1′ 20″		15	30	—
	Washing with Tyrode's solution.			
Before		15—16	30	—
	adrenaline			
Promptly			2	— 1
After				
40″		15	22	—
1′		15—16	30	

causes a momentary decrease of the amplitude from 20 mm. to 1 mm. After 30—40 seconds the amplitude is again 20 mm. The frequency does not change during this period. After washing, 20 ml. lead trypan blue was added which in this experiment gives a less marked self-effect. After the kymograph was stopped for 10 min., the amplitude was controlled for appr. 15 min. and after the above-mentioned addition of 20 ml., the same amount of adrenaline was added, whereby a momentary decrease of the amplitude was obtained; then it slowly increased and after 5 min. it had only reached 15 mm. Tonus decreased temporarily to 0.5—1.0 mm. After washing, adrenaline was added whereby a momentary decrease of the amplitude from 28 mm. to 2 mm. with a simultaneous tonus decrease to 0.5—1 mm was obtained. After 1 min. the amplitude was again 28 mm. After another washing and addition of adrenaline, a momentary decrease of the amplitude to 2 mm. with a decrease of tonus to 0.5—1 mm was obtained. After 1 min. the amplitude was 30 mm.

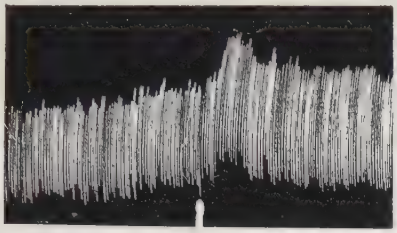
When testing the effect of lead trypan blue on the muscular function by adding $BaCl_2$ no difference was found.

Previous examinations of the effect of thorium nitrate on surviving organs seem to have been made only by Sollmann & Brown (1905). They tested the effect of thorium nitrate on an intestine mounted in a Locke-solution. Any attempts to compare the effect of pharmacon in this connection was of course not made. 10 cm. long intestine pieces were placed in a Locke-solution at 38° C, through which was passed an oxygen flow. Three series of experiments lead to the following conclusions; thorium-sodiumnitrate causes vermicular movements followed by tonic contraction. When using thorium nitrate this coagulates (1 % solution). The test of the effect of thorium trypan blue on surviving organs is therefore of certain interest. Also in this case, the solution which was added to the glass cylinder was made of equal amounts of saturated thorium trypan blue and double Tyrode's solution. The thorium content corresponds to 1.79 mg % thorium. An addition of 10 ml. to the glass cylinder corresponds to a thorium nitrate solution of 1 : 900,000. Thus it is a case of extremely small amounts.

Thorium trypan blue has already, when 2 ml. are added, a slight effect on the intestine which becomes clear with 5, and pronounced with an addition of 10 —20 ml. As an example (Fig. 3), the following experiment will be given: the addition of 10 ml. thorium trypan blue gave within 20 seconds a tonus increase of 8 mm., whereby the amplitude increased from 15 to 20 mm. After 4—5 min. the tonus was once again at the starting value while the amplitude still remained at 20 mm. Gradually the amplitude decreases to values below the starting value. The frequency remained unaltered the whole time.

The following result (Fig. 4) was obtained from the experiments on the parasympathetic irritability in the intestine when under the influence of therium trypan blue. With amounts up to 5 ml. no certain effect can

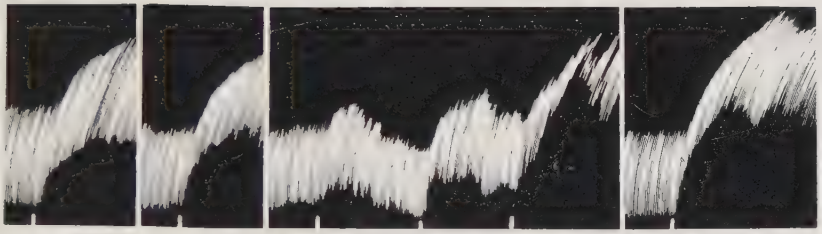
Fig. 3. Intestine of rabbit.



Th

Th = 10 ml. thoriumtrypan blue.

Fig. 4. Intestine of rabbit.



Ba x Pil x Th Pil Ba x Pil

Ba = 0.3 ml. 0.2 % $BaCl_2$.
Pil = 0.3 ml. 0.02 % Pilocarpine
Th = 20 ml. Thoriumtrypan blue.
x = washing with Tyrode's solution.

be determined. With 10 ml. it becomes clear and with 20 ml. a more tangible inhibition of pilocarpine effect is observed. As an example the following experiment must be mentioned: the addition of 0.3 ml. $BaCl_2$ gives an almost momentarily increase of tonus, which during 0—1 min. increases up to + 15 mm. so as to be for 3—4 min. 23 mm. above the starting value. Simultaneously, the amplitude increases from 30 to 34—35 mm. The frequency has increased insignificantly (from 16 to 17 beats per min.). After washing 0.25 ml. 0.01 % pilocarpine solution was added, whereby the tonus increased momentarily during the first minute to + 18 mm. so as to be at + 28 mm. for 3—4 min. The amplitude increased gradually from 25 mm. to 30 mm. The frequency increased from 12 to 15 beats per min. After washing, 20 ml. thorium trypan blue was added, whereby the tonus increased to 8 during the first minute, then decreased during 1—2 min. to + 5 and decreased so that 4 min. after the addition it was 3 mm. below the starting value. The amplitude increased during the first minute from 25 to 27 mm., and decreased so that 2 min. after the addition it was 22 mm. It is not certain whether the frequency

Table 6. *Intestine of rabbit* ($^{14}/_{11}$ *1941*). (Fig. 4.)

Time	Addition	Frequency/min.	Oscillation size in mm.	Tonus in mm.
	Preparation 1. Thorium trypan blue + pilocarpine + $BaCl_2$.			
Before		16	30	—
	0.3 ml. $BaCl_2$			
After				
0—1′		17	34—35	+ 15
3—4′		17	34—35	+ 23
	Washing with Tyrode's solution.			
Before		12	25	—
	0.25 ml. pilocarpine			
After				
0—1′		10—11	25	+ 18
3—4′		15	30	+ 28
	Washing with Tyrode's solution.			
Before		17	25	—
	20 ml. thorium trypan blue			
After				
0—1′		18	25—27	+ 8
1—2′		18	22	+ 5
4′			22	— 3
	pilocarpine			
After				
0—1′		18	22	+ 16
3—4′		16	22—25	+ 10—12
	$BaCl_2$			
After				
0—1′			about 25	+ 24 (= 12 + 12)
3—4′			about 25	+ 67 (= 12 + 45)
	Washing with Tyrode's solution.			
Before		15	17	—
	pilocarpine			
After				
1′				+ 24
3—4′		18—20	16	+ 25—30
	Preparation 2. Self-effect. (Fig. 3.)			
Before		17	17	—
	10 ml. thorium trypan blue			
After				
0—1′		18	22	+ 5
3—4′		20	20	—

remains unchanged. Pilocarpine was now added whereby the tonus after 1 min. was 16 mm., and after 3—4 min. 22—25 mm. The frequency decreased from 18—16 beats per min. 8 min. after the addition of thorium trypan blue, $BaCl_2$ was added whereby the tonus increased to appr. + 25 mm. (12 + 12) during 0—1 min. Arter 3—4 min. the tonus was + 67 mm. (12 + 45). Amplitude and frequency were unchanged. After washing, pilocarpine was added whereby the tonus during 1 min. increased to + 24 mm., and after 3—4 min. to + 25—30 mm. The amplitude decreased from 17 to 16 mm. The frequency increased from 18 to 18—20 per min.

In experiments with adrenaline it has not been able to prove any clear effect of thorium trypan blue on the reaction of the intestine to this just mentioned irritation.

Nor does thorium trypan blue seem to have any influence on a subsequent addition of $BaCl_2$.

When making blood pressure tests on rabbits, it was impossible to prove any effect of injections with up to 50 ml. saturated solution in physiologic salt on the blood pressure.

CHAPTER V.

The Effect of Lead Trypan Blue on Cancer in White Mice, Induced by Tar-Painting.

1. The occurrence of the tumours.

In this examination the experiment with lead trypan blue begins firstly when the animals have definitely developed a tumour. Therefore, it is to be expected that test and control animals behave similarly during the time which elapsed from the beginning of the painting to the day when the tumour first appeared.

This table shows a certain skewness of the material, the tumour seeming to develop earlier in test than in control animals; this is because the first tumours developed earlier than was expected. As it was impossible to treat them the first days after the onset of the tumour, some of the tumour-bearing animals which were, from the beginning, intended as test animals were transferred to the control animals (3 males and 2 females). This change does not otherwise seem to have caused any influence on the combination of the material. It seems as if the test males developed tumours more often than the control males, but the difference is 6.3 $\pm$ 4.7 per cent and thus not statistically proved. The difference

Table 7. *Number of tumours occurring during different periods in proportion to the number of tar-painted animals.*

		Weeks	−8	−10	−12	−14	−16	−18	−20	−22	−24	−26	−28	−30	>30	Total
Test animals	♂	Obs.*	—	—	—	4	18	11	11	7	2	2	2	—	1	58
		%*	—	—	—	2.1	9.5	5.8	5.8	3.7	1.1	1.1	1.1	—	0.5	30.7
	♀	Obs.	—	—	2	9	21	13	10	10	4	3	1	3	2	78
		%	—	—	1.1	5.0	11.7	7.2	5.6	5.6	2.2	1.7	0.6	1.7	1.1	43.3
	♂ ♀	Obs.	—	—	2	13	39	24	21	17	6	5	3	3	3	136
		%	—	—	0.5	3.5	10.6	6.5	5.7	4.6	1.6	1.4	0.8	0.8	0.8	36.9
Control animals	♂	Obs.	2	—	3	5	8	3	9	4	2	4	—	—	2	42
		%	1.2	—	1.7	2.9	4.7	1.7	5.2	2.3	1.2	2.3	—	—	1.2	24.4
	♀	Obs.	1	1	4	7	15	9	9	6	4	2	1	—	3	62
		%	0.6	0.6	2.6	4.5	9.7	5.8	5.8	3.9	2.6	1.3	0.6	—	1.9	40.3
	♂ ♀	Obs.	3	1	7	12	23	12	18	10	6	6	1	—	5	104
		%	0.9	0.3	2.1	3.7	7.1	3.7	5.5	3.1	1.8	1.8	0.3	—	1.5	31.9

* Obs. = Observed number with tumour. % = the tumours in per cent of painted animals.

between test and control animals is 5.8 ± 3.6. Thus it appears from the table that apart from the above mentioned skewness in the number of early formed tumours, the distribution of the origin of tumours during different periods after the beginning of the tar-painting is equal in test and control animals. The *females developed tumours more often than the males*, and the difference between test females and test males becomes 12.6 ± 5.0 per cent, and is thus statistically probable;

the difference between control females and control males becomes 15.9 ± 5.1 per cent, and is thus statistically significant;

the difference between females (test and control animals) *and males* (test and control animals) *becomes 14.8 ± 3.6, and thus statistically significant.*

That time which averagely elapsed from the beginning of the tar-painting to the beginning of tumour formation is for

57 lead trypan blue treated males..... 130.2 ± 4.3 days
76 » » » » females..... 130.3 ± 4.0 »
43 non-treated males ..... 133.7 ± 8.0 »
61 » females..... 129.5 ± 7.2 »

It appears from this, that the time required to produce tumours as counted from the day the tar-painting began is equally long for test and control animals, and for males and females.

Table 8. Series I, III. *Number of tumours and surviving animals at varying periods from the beginning of the tar-painting. The tumours are given in per cent of the total number of painted animals.*

	Weeks		0	2	4	6	8	10	12	14	16	18	20	22	24	26	28	30	32	34	36	38	40
Test animals	♂	*Obs.	189	156	119	104	100	91	87	85	75	70	68	57	42	34	26	21	19	15	8	6	4
		No.	—	—	—	—	—	—	—	6	21	29	39	43	33	28	23	18	16	12	7	5	3
		%	—	—				—	—	3.2	11.1	15.3	20.6	22.8	17.4	14.8	12.2	9.5	8.5	6.3	3.7	2.6	1.6
	♀	*Obs.	180	157	136	126	120	113	104	100	94	90	86	78	72	68	60	47	37	31	24	21	14
		No.	—	—	—	—	—	—	1	7	29	36	49	58	55	52	48	38	30	25	20	18	12
		%	—	—	—	—	—	—	0.6	3.9	16.1	20.0	27.2	32.2	30.6	28.9	26.7	21.1	16.7	13.9	11.1	10.0	6.7
	♂+♀	*Obs.	369	313	255	230	220	204	191	185	16.9	160	154	135	114	102	86	68	56	46	32	27	18
		No.	—	—	—	—	—	—	1	13	50	65	88	101	88	80	71	56	46	37	27	23	15
		%	—	—	—	—	—	—	0.3	3.5	13.6	17.6	23.8	27.4	23.8	21.7	19.2	15.2	12.5	10.0	7.3	6.2	4.1
Control animals	♂	*Obs.	172	144	120	106	93	90	68	66	61	55	52	47	43	32	28	26	23	12	10	8	5
		No.	—	—	—	1	1	1	3	9	17	20	28	29	30	26	23	21	18	8	6	6	3
		%	—	—	—	0.6	0.6	0.6	1.7	5.2	9.9	11.6	16.3	16.9	17.4	15.1	13.4	12.2	10.5	4.7	3.5	3.5	1.7
	♀	*Obs.	154	139	128	121	112	103	100	94	78	74	66	64	55	45	39	35	29	23	16	16	13
		No.	—	—	—	—	—	2	6	12	28	34	42	49	44	36	32	28	22	17	10	11	8
		%	—	—	—	—	—	1.3	3.9	7.8	18.2	22.1	27.3	31.8	28.6	23.4	20.8	18.2	14.3	11.0	6.5	7.1	5.2
	♂+♀	*Obs.	326	283	248	227	205	193	168	160	139	129	118	111	98	77	67	61	52	35	26	24	18
		No.	—	—	—	1	1	3	9	21	45	54	70	78	74	62	54	49	40	25	16	17	11
		%	—	—	—	0.3	0.3	0.9	2.8	6.4	13.8	16.6	21.5	23.9	22.7	19.0	16.6	15.0	12.3	7.7	4.9	5.2	3.4

* Obs. = observed number. No. = number of tumours. % = per cent tumours.

In table 8 appears the number of surviving, tumour-bearing animals during different periods from the beginning of the experiment in proportion to the number of tar-painted animals. Also here it is evident, that the figures for the females are higher than for the males. When comparing between females and males, the tumour frequency calculated on the number of tar-painted animals becomes as follows:

Table 9.

	Number of weeks after the beginning of tar-painting	Difference between males and females
Test animals	22	9.4 ± 4.6 % not proved
	24	13.2 ± 4.4 % significant
	26	14.1 ± 4.3 % significant
Control animals.....	22	14.9 ± 4.7 % significant
	24	11.2 ± 4.6 % not proved
	26	8.3 ± 4.4 % not proved
	18	7.4 ± 2.9 % probable
Test animals	22	12.1 ± 3.4 % significant
	24	12.1 ± 3.2 % »
Control animals	26	11.3 ± 3.1 % »
	28	11.3 ± 2.9 % »

Table 10 shows the number of surviving animals in proportion to the number of tar-painted animals. At 4 and 6 weeks after the beginning of tar-painting the figures for the test animals are lower. After 4 weeks the difference is 6.3 ± 3.4 per cent, and is not proved. After 6 weeks the difference is 7.3 ± 3.6 per cent, and is likewise not proved. Besides this no noteworthy difference between control and test animals exists. On the other hand, the figures for the females are considerably higher than for the males during the same period of time, and this is true of the test as well as the control animals. When comparing between females and males, the frequency of the surviving, in proportion to the number of tar-painted animals is as follows:

From the diagram 1 appears that mortality in the females is lower than that in the males during the tar-painting period, as well as after they had developed tumours.

As appears from the table a certain increase in the mortality exists in the test males during the 6th —8th week as compared to the control males. The difference between test and control males in the 6th week is 4.1 ± 2.0 per cent and is thus not proved; when conjoining the 6th and 8th week the difference becomes 6.6 ± 2.6 per cent, and is thus statistically probable. This increased mortality in the beginning of the experiment should be due to a toxic influence of the injections. If one studies

Table 10. Series I, III. *Number surviving animals at varying periods given in per cent of the number of the painted animals.*

	Weeks		0	2	4	6	8	10	12	14	16	18	20	22	24	26	28	30	32	34	36	38	40
Test animals	♂	* Obs.	189	156	119	104	100	91	87	85	75	70	68	57	42	34	26	21	19	15	8	6	4
		Surv.	100.0	82.5	63.0	55.0	52.9	48.1	46.0	45.0	39.7	37.0	36.0	30.2	22.2	18.0	13.8	11.1	10.1	7.9	4.2	3.2	2.1
	♀	* Obs.	180	157	136	126	120	113	104	100	94	90	86	78	72	68	60	47	37	31	24	21	14
		Surv.	100.0	87.2	75.6	70.0	66.7	62.8	57.8	55.6	52.2	50.0	47.8	43.3	40.0	37.8	33.3	26.1	20.6	17.2	13.3	11.7	7.8
	♂+♀	* Obs.	369	313	255	230	220	204	191	185	169	160	154	135	114	102	86	68	56	46	32	27	18
		Surv.	100.0	84.8	69.8	62.3	59.6	55.3	51.8	50.1	45.8	43.4	41.7	36.6	30.9	27.6	23.3	18.4	15.2	12.5	8.7	7.3	4.9
Control animals	♂	* Obs.	172	144	120	106	93	90	68	66	61	55	52	47	43	32	28	26	23	12	10	8	5
		Surv.	100.0	83.7	69.8	61.6	54.1	52.3	39.5	38.4	35.5	32.0	30.2	27.3	25.0	18.6	16.3	15.1	13.4	7.0	5.8	4.7	2.9
	♀	* Obs.	154	139	128	121	112	103	100	94	78	74	66	64	55	45	39	35	29	23	16	16	13
		Surv.	100.0	90.3	83.1	78.6	72.7	66.9	64.9	61.0	50.6	48.1	42.9	41.6	35.7	29.2	25.3	22.7	18.8	14.9	10.4	10.4	8.4
	♂+♀	* Obs.	326	283	248	227	205	193	168	160	139	129	118	111	98	77	67	61	52	35	26	24	18
		Surv.	100.0	86.8	76.1	69.6	62.9	59.2	51.5	49.1	42.6	39.6	36.2	34.0	30.1	23.6	20.6	18.7	16.0	10.7	8.0	7.4	5.5

* Obs. = Observed number of animals. Surv. = Surviving animals in per cent.

Table 11.

	Number of weeks after beginning of tar-painting	Difference
Test animals	6	15.0 ± 5.0 % significant
	10	14.7 ± 5.1 % probable
	14	10.6 ± 5.2 % not proved
	18	13.0 ± 5.1 % probable
	24	17.8 ± 4.7 % significant
	30	15.0 ± 4.0 % significant
Control animals....	6	17.0 ± 5.0 % significant
	10	14.6 ± 5.4 % probable
	14	22.6 ± 5.4 % significant
	18	16.1 ± 5.4 % almost significant
	24	10.7 ± 5.0 % not proved
	30	7.6 ± 4.3 % not proved

Table 12. *Number of dead animals in proportion to the number of tar-painted animals from the beginning of the experiment (= at different tumour-ages).*

Weeks			−2	−4	−6	−8	−10	−12	−14	−16	−18	−20	−22	−24	−26	−28	>28
Test animals	♂	Numb.*	2	3	11	8	6	4	3	3	2	8	—	2	1	—	—
		%*	1.1	1.6	5.8	4.2	3.2	2.1	1.6	1.6	1.1	4.2	—	1.1	0.5	—	—
	♀	Numb.	1	3	4	7	7	9	9	8	9	4	2	2	3	—	3
		%	0.6	1.7	2.2	3.9	3.9	5.0	5.0	4.4	5.0	2.2	1.1	1.1	1.7	—	1.7
Control animals	♂	Numb.	1	1	3	3	5	7	3	3	6	4	4	—	—	—	—
		%	0.6	0.6	1.7	1.7	2.9	4.1	1.7	1.7	3.5	2.3	2.3	—	—	—	—
	♀	Numb.	3	2	3	3	12	13	4	8	5	3	1	1	1	1	1
		%	1.9	1.3	1.9	1.9	7.8	8.4	2.6	5.2	3.2	1.9	0.6	0.6	0.6	0.6	0.6

* Numb. = Number-tumour bearing animals. % = the tumour animals in per cent of painted animals.

the figures concerning the higher tumour-ages, no significant difference is found between the males, and for the females no significant difference as to mortality during the experiment is found.

Vannfält considers that he has proved that the age of the test animals at the beginning of the tar-painting is of no importance to the origin of the tumour, also that the weight of the animal at that time seems to be of no influence. As the above stated figures, concerning the mortality at different periods, show accordance between test and control animals, and because they are divided from the beginning into different groups from different batches, it can be assumed that the age and weight

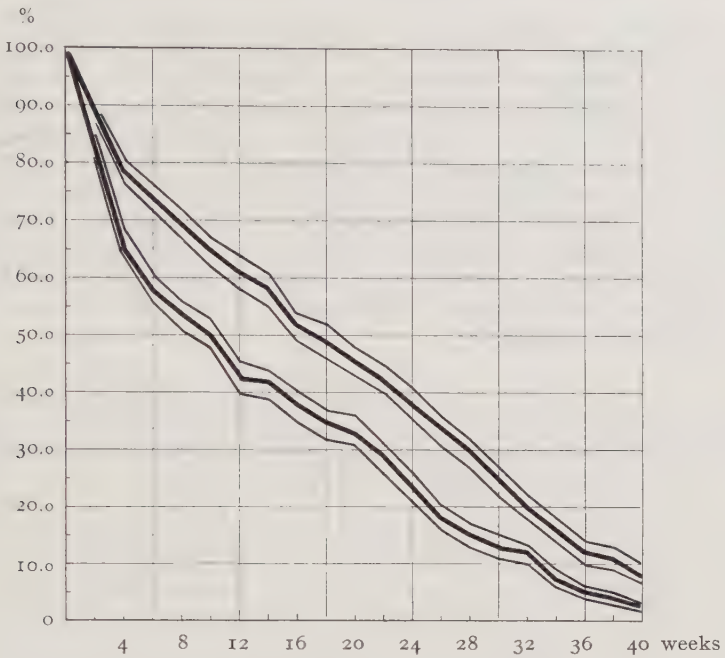


Diagram 1. The number surviving animals at different periods in relation to the painted number animals. The figures are given in per cent with one time standard error. The upper curve = females (tests and controls). The lower curve = males [tests and controls). Series I, III.

of the test and control animals is on an average equal. It is therefore of no interest to account for these figures.

Summarizing, it appears from the above mentioned that the test and control animals are similar, in so far that they both required an equally long time to develop tumours, and both groups have the same tendency to develop tumours. That difference as regards continued duration of life from that moment the animal developed tumour (in the following called tumour-age), the tumour growth and metastasizing tendency which is proved in the following must accordingly be the result of the treatment with lead trypan blue.

If one calculates the average tumour-age of those animals that died spontaneously, it is found to be

for 42	males treated with lead trypan blue.....	77.5 ± 6.3	days
35	non-treated males.....	94.2 ± 6.1	»
60	females treated with lead trypan blue.....	90.0 ± 5.3	»
57	non-treated females.....	82.7 ± 5.0	»

The difference between test and control animals is 7.3 ± 7.3 days. The average tumour-age of the female mice is thus not affected which was not to be expected either in view of the results of the toxicity test. Among the male mice, the control animals have, on an average, a greater tumour-age than the test animals; the difference is 16.7 ± 8.8 days and it is thus not significant. When performing the toxicity examination it appeared that normal males treated with lead trypan blue had a statistically significant shorter life than the control animals. The numerically shorter tumour-age of the control males stated above can possibly depend upon the fact that the treatment with lead trypan blue through its influence on the tumours compensates, to some extent, its otherwise life-shortening influence.

Table 13. *The average body-weight at different periods after the beginning of the test (= at different tumour ages).* Series I, III. Females.

Weeks	Non-treated females			Treated females			Diff.
	n	$M \pm \varepsilon(M)$	σ	*n*	$M \pm \varepsilon(M)$	σ	
0	60	21.57 ± 0.62	4.82	68	22.57 ± 0.54	4.49	1.00 ± 0.82
2	56	21.84 ± 0.73	5.45	65	22.75 ± 0.54	4.38	0.91 ± 0.91
4	54	22.33 ± 0.81	5.99	62	22.96 ± 0.57	4.50	0.63 ± 0.99
6	48	23.53 ± 0.89	6.20	56	23.08 ± 0.59	4.39	− 0.45 ± 1.07
8	38	24.36 ± 1.10	6.78	48	23.63 ± 0.70	4.85	− 0.73 ± 1.30
10	24	24.98 ± 1.53	7.47	34	25.19 ± 0.92	5.35	0.21 ± 1.79
12	18	26.26 ± 1.74	7.40	27	25.48 ± 1.16	6.02	− 0.78 ± 2.09
14	11	28.11 ± 2.16	7.18	16	26.06 ± 1.53	6.11	− 2.05 ± 2.65
16	10	28.23 ± 1.93	6.11	12	26.31 ± 1.69	5.87	1.92 ± 2.57
18	7	29.32	—	9	28.50	—	
20	4	30.06	—	7	28.11	—	

Table 14. *The average body-weight at different periods after the beginning of the test (= at different tumour ages).* Series I, III. Males.

Weeks	Non-treated males			Treated males			Diff.
	n	$M \pm \varepsilon(M)$	σ	*n*	$M \pm \varepsilon(M)$	σ	
0	40	24.36 ± 0.90	5.71	53	22.74 ± 0.45	3.25	− 1.62 ± 1.01
2	39	24.17 ± 0.92	5.73	51	22.78 ± 0.46	3.25	− 1.39 ± 1.03
4	38	24.33 ± 0.93	5.71	43	23.08 ± 0.59	3.85	− 1.25 ± 1.10
6	33	25.27 ± 1.09	6.28	33	23.07 ± 0.65	3.71	− 2.20 ± 1.27
8	25	25.52 ± 1.32	6.58	24	24.07 ± 0.74	3.63	− 1.45 ± 1.51
10	17	27.82 ± 1.50	6.18	16	24.30 ± 0.91	3.63	− 3.52 ± 1.75
12	13	27.19 ± 2.03	7.32	13	24.67 ± 1.00	3.61	− 2.52 ± 2.26
14	10	28.53 ± 2.39	7.55	8	25.66	—	
16	7	30.86	—	5	27.40	—	
18	6	32.58	—	4	28.50	—	
20	2	38.00	—	2	30.75	—	

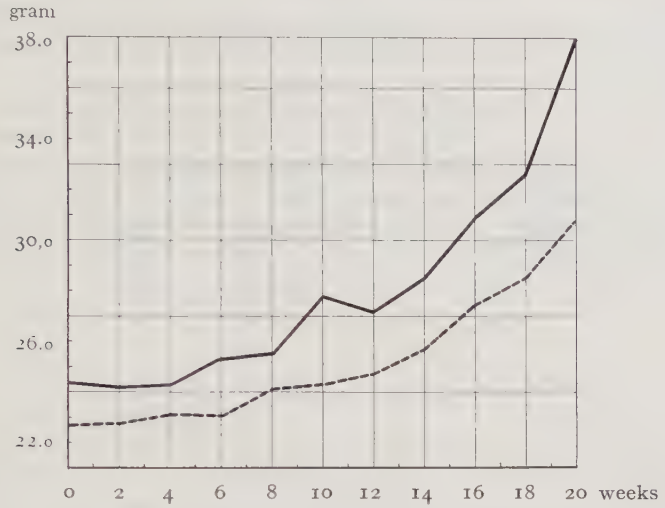


Diagram 2. The average body-weight at different periods from the beginning of the test (= at different tumour age). Series I, III.

——— = non-treated males - - - - - - - - = treated males

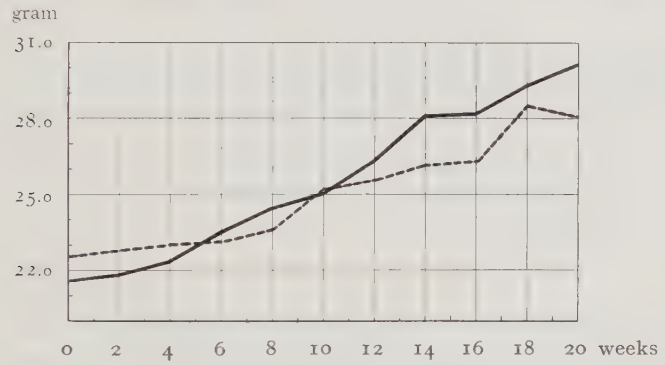


Diagram 3. The average body-weight at different periods from the beginning of the test (= at different tumour age). Series I, III.

——— = non-treated females - - - - - - - - = treated females

As has been shown previously, treatment with leadtrypan blue yielded no provable shortening of the length of life of the tumour-bearing animals as reckoned from the beginning of the experiment. In order to see whether the body-weight had altered due to the treatment as a symptom of the treatment's effect on the general condition of the animal, the body-weight with standard error including the curves

have been given in the table above. From the tables and curves it becomes clear that the treatment has had no effect on the animals in this respect.

The given lead trypan blue doses.

When treating with lead trypan blue the animals were given subcutaneous injections three times a week corresponding to 2 per cent of the body weight each time. Calculated in metallic lead the given doses were as follows

males 0.60 ± 0.06 mg. ($n = 57$)
females 0.78 ± 0.07 mg. ($n = 76$)

The rate of tumour growth.

During the course of the investigation, the size of the tumour has been registered each time it was weighed, and it was stated as being as big as a millet seed, a grain of rice and a pea etc. In order to obtain a more exact value, the dissected tumour was weighed with an accuracy of 0.01 gram at the obduction. Even though the estimation of the tumour size in the living animal becomes relatively accurate, according to VANNFÄLT, it is, however, difficult through estimation to obtain a reliable conception as to the tumour-growth. In order to find out whether the treatment with lead trypan blue has had any influence on this, the tumour growth per day has been utilized as an approximate value; this value was ascertained by dividing the tumour weight at the obduction with the number of days during which the tumour was observed with certainty. The mean value of this tumour growth per day has then been calculated for those animals that have had a tumour for at least 30 and 60 days respectively. The results appear from the table below.

Table 15. *Tumour growth per day in lead trypan blue-treated animals, and in normal non-treated animals.*

Sex	Treated with lead trypan blue		Control animals	
	Number	$M \pm \varepsilon(M)$	Number	$M \pm \varepsilon(M)$
	Tumour-age, at least 30 days			
Males	41	16.5 ± 2.4 mg.	31	30.5 ± 9.0 mg.
Females	51	16.9 ± 2.5 mg.	51	26.1 ± 3.9 mg.
Both sexes	92	16.6 ± 1.7 mg.	82	27.7 ± 4.1 mg.
	Tumour-age, at least 60 days			
Both sexes	70	17.7 ± 1.6 mg.	72	27.1 ± 3.3 mg.

As can be seen from the table, the *tumour growth per day is greater in the control male and female mice, than in the test male and female mice* The difference between control and test animals is, in animals with a tumour-age of at least 30 days, 11.1 ± 4.4 mg. per day, and in animals with a tumour-age of at least 60 days 9.4 ± 3.6 mg. per day. The average tumour-age of at least 30 days in the control animals is 93.3 ± 3.9 days, and in the test animals 92.2 ± 3.8 days, and of at least 60 days for control animals 100.4 ± 3.8 days, and for test animals 106.0 ± 3.9 days. Thus as the tumour-age is equal in control and test animals, then *the difference as to the average tumour growth becomes statistically probable, and almost significant.*

When calculating the average tumour weight of the observed tumours the following values are obtained:

test males	1.44 ± 0.21	gram
test females	1.57 ± 0.22	»
test males − test females	1.51 ± 0.15	»
control males	2.35 ± 0.51	»
control females	2.28 ± 0.34	»
control males − control females	2.31 ± 0.29	»

The control animals thus seem to have higher values for the average tumour growth than the test animals. The difference becomes:

males	0.91 ± 0.55 gram	not proved
females	0.71 ± 0.40 gram	not proved
males + females	0.80 ± 0.32 gram	statistically probable

It is conceivable that this difference in weight depends on the fact that the control animals died, or were killed, later than the test animals. Therefore it is necessary to examine the conditions in this respect.

The average tumour-age for the same tumours are:

test males	81.6 ± 6.4	days
test females	91.3 ± 5.8	»
test males + test females	87.1 ± 4.3	»
control males	92.0 ± 6.6	»
control females	87.2 ± 5.6	»
control males + control females	89.0 ± 4.3	»

No important difference exists between test and control animals. The difference is 1.9 days. No noteworthy difference is found either between test and control males, or between test and control females.

Further there is the possibility of the effect of the treatment on the tumour itself. It can be thought that the tumours might be inhibited as regards malignancy. If such a difference is found, then it could explain the difference in the tumour weight, as malignancy signifies a quicker proliferation. In table 16 the number of benignant tumours at different tumour-ages is given. If one calculates the frequency of benignant tumours from the total number of tumours at a tumour-age exceeding 50 days, the number of benignant tumours in the test animals is 8.7 per cent, and in the control animals 2.4 per cent. The difference between the test and control animals becomes 6.3 ± 3.4 per cent, thus the difference is not proved.

The diagonis of the tumours.

Most of the tumours were cancer but in this material 20 cases with tumours were found in which both carcinoma and sarcoma-like tissue were seen. These tumours were considered as carcinoma. Two tumours were spindle cell sarcoma and of these one gave sarcomametastasis to a lymph node. These two cases have also been included when treating the material.

2. Metastasizing.

The microscopic picture shows as a rule, whether a tumour is malignant or not. The occurrence of metastases is of great significance especially in order to determine the diagnosis, if this, as regards the appearance of the primary tumour is somewhat uncertain. In my material there were one or two cases where a small tumour, which judging from the microscopic preparation could be benignant, but in which malignancy could be determined because of the existence of metastases. When judging the result of the treatment of tumours, it is therefore important also to consider whether the tumours' distribution has possibly become inhibited. Table 16 shows a survey over the metastasizing of tumours to the lymph nodes and internal organs. For further details I refer to those tables of the entire research material in the University Library in Uppsala. Metastasizing seems to begin at a tumour-age of 50 days. If, according to the standard method, one calculates the number of test animals which should develop metastases after 50 days, as compared to the control animals, the figures in table 17 are obtained:

Tables 16 a—d. *Table of the incidence of metastases at varying tumour-age.*

Table 16 a. Series I, III. Males, treated with lead trypan blue.

Days	0—30	31—60	61—90	91—120	121—150	>150	Total
benign. tumour	4	3	2	—	—	1	10
tumour without metastasis	3	13	7	4	7	1	35
tumour with metastases of the lymph nodes only	—	1	—	1	1	—	3
tumour with metastases of the lungs only	—	—	1	1	—	—	2
tumour with metastases in two sites	—	—	—	1	1	1	3
tumour with metastases in more than two sites	—	—	—	—	—	—	—
total	7	17	10	7	9	3	53

Table 16 b. Series I, III. Males, non-treated.

Days	0—30	31—60	61—90	91—120	121—150	>150	Total
benign. tumour	1	1	—	—	1	—	3
tumour without metastasis	2	2	8	1	1	—	14
tumour with metastases of the lymph nodes only	—	2	3	3	3	—	11
tumour with metastases of the lungs only	—	—	—	1	1	—	2
tumour with metastases in two sites	—	—	—	3	5	1	9
tumour with metastases in more than two sites	—	—	1	—	—	—	1
total	3	5	12	8	11	1	40

Table 16 c. Series I, III. Females, treated with lead trypan blue.

Days	0—30	31—60	61—90	91—120	121—150	>150	Total
benign. tumour	1	1	1	1	—	2	6
tumour without metastasis	4	10	10	7	2	3	36
tumour with metastases of the lymph nodes only	—	—	3	5	6	1	15
tumour with metastases of the lungs only					—		—
tumour with metastases in two sites	—	—	1	2	2	—	5
tumour with metastases in more than two sites	—	—	4	—	2	—	6
total	5	11	19	15	12	6	68

Table 16 d. Series I, III. Females, non-treated.

Days	0— 30	31— 60	61— 90	91— 120	121— 150	>150	Total
benign. tumour	—	1	1	—	—	—	2
tumour without metastasis	6	4	13	5	1	2	31
tumour with metastases of the lymph nodes only	—	—	7	6	—	—	13
tumour with metastases of the lungs only	—	—	1	1	—	—	2
tumour with metastases in two sites	—	—	4	1	3	2	10
tumour with metastases in more than two sites	—	—	1	1	1	—	3
total	6	5	27	14	5	4	61

Table 17. *The number of metastases in test animals as compared to that number which should have existed if they had been subjected to the same risks as the control animals.*

Animals treated with lead trypan blue	Observed number with metastatis	Calculated number with metastasis	Difference
Males	8 ± 2.60	20.35 ± 2.44	12.35 ± 3.57
Females	25 ± 3.72	29.68 ± 3.63	4.68 ± 5.20
Males + females	35 ± 4.55	50.20 ± 4.48	17.20 ± 6.38

Treatment with lead trypan blue thus inhibits the metastazing in males, so that the difference between control males and test males becomes 12.35 ± 3.57, *and is thus statistically significant. In the females on the contrary, the treatment appears to entail no effect as the difference is* 4.68 ± 5.20.

a. *Metastasizing to the lymph nodes.*

The most common metastasizing regions for tar tumours in mice are the lymph nodes, regional in relation to that part of the skin which was painted. The blue-black, striped-like pigment, possibly caused by lead trypan blue, which can sometimes be observed macroscopically, is not seen in the microscopic picture as it has probably been dissolved when staining the preparation. In the majority of the cases it can already be seen macroscopically whether lymph nodes are the seat of metastasizing, or infiltrated growth inwards of the tumour tissue from the surroundings.

When calculating the frequency of the metastases which would have existed in the treated animals, if they had been subjected to the same risks as the control animals, the following figures are obtained:

Table 18.

Number of animals treated with lead trypan blue	Observed number with metastases	Calculated number with metastates	Difference
Males	6 ± 2.10	24.62 ± 4.21	18.62 ± 4.72
Females	25 ± 3.31	24.51 ± 3.45	0.49 ± 4.78

Thus the treatment with lead trypan blue has inhibited the metastasizing to the regional lymph nodes. The difference between control and test males is 18.62 ± 4.70, and thus more than three times its own standard error, and so statistically significant; contrary to this the treatment had no effect on the females.

The regional lymph nodes are, as is known, four in number. Most often it is one or two that are the site of metastasizing, seldomly three or four. If one attempts to group the distribution of metastases so that one group comprises metastases to only one or two lymph nodes, and the other group comprises metastases to one or more lymph nodes and to another organ, then the following distribution is found.

Table 19.

	Number with tumour	Number with more than two lymph node metastases	% cases with more than two lymph node metastases etc.
Test males	31	4	12.9
Test females	53	9	17.0
Males + females	84	13	15.5
Normal males	32	12	37.5
» females	49	16	32.7
Males + females	81	28	34.6

When comparing between control and test animals the difference becomes 24.4 ± 10.5 per cent, and is thus not proved. The difference between control animals (males and females) is 19.1 ± 6.6 per cent, and almost statistically significant.

b. *Metastasizing to the lungs.*

Next to the lymph nodes, the lungs are the most common region for metastases originating from tumours on the skin of the backs of mice. In 6 cases metastases existed in the lungs without simultaneous lymph node metastases (see table 16).

Table 20. *The frequency of lung metastases at different tumour-age.*

Tumour-age in days		61—90	91—120	121—150	> 150	Total	Per cent of the number of animals with malignant tumour
Treated* ...	♂	1	2	1	1	5	5/44 = 11.4 } 13.2
	♀	4	2	3	—	9	9/62 = 14.5 }
Non-treated	♂	2	3	4	1	10	10/36 = 27.8 } 25.3
	♀	6	2	4	2	14	14/59 = 23.7 }

* With lead trypan blue.

From the table it appears as if lung metastases were more frequent in the non-treated mice than in the treated ones. The difference is 11.5 ± 6.9 and is thus not proved, which is why it is of significance only if the difference points in the same direction as the other results.

From a pathological-anatomical viewpoint, it is a question of cancroid in all the cases. In this examination no sarcomatous lung metastases were found.

In the non-treated mice, metastasis to the hilus lymph nodes is found in 2 cases.

Lung adenomae are also found in this examination; they are a form of benignant spontaneous tumours and their presence is dependent on the susceptibility of the employed mice strain to develop tumours, and also on their age. It can be seen from the table that lung adenomae are evenly distributed in the control and test animals.

Table 21. *The incidence of lung adenoma in tumour-bearing animals.*

Tumour-age in days		31—60	61—90	91—120	121—150	> 150	Total
Treated*	♂	1	1	—	2	1	5
	♀	—	1	3	—	2	6
Non-treated	♂	1	—	—	1	—	2
	♀	—	4	5	—	—	9

* With lead trypan blue.

c. *Metastasizing to other internal organs.*

In no cases signs of metastases in the heart have been found. Sometimes a lung tumour grew out to the mediastinum and, through this, it was able to grow over the external layer of the parietal pericardium. However, one has not been able to observe any cancer of the internal layer of the pericardium, and in any case not on the heart itself. In the remaining internal organs some few metastases occur, namely 1 in the liver, 2 in the kidneys and 2 in the milt, all female cases.

3. Analysis of lead content in tissue.

Some authors have stated that lead should have a certain affinity for tumour tissue (Bell etc.), this fact has, however, been doubted by others. When these experiments with lead trypan blue were carried out, no sufficiently sensitive methods for the determination of lead content in biological material existed. During recent years, however, the method has been essentially improved. The examination is carried out with semiquantitative spectral analysis by means of intermittent electric arc (method according to K. Pfeilsticker). The ash from the liver of one mouse contained 0.006—0.004 per cent lead and the tumour 0.015—0.010 per cent. An analysis of the ash from the testicles of 13 mice showed a lead content of 0.008—0.005 per cent. As the mouse used for the analysis of the lead content in the tumor and liver had received 1.48 mg. metallic lead on 22 gram body weight, while the mice used for the later analysis had received 9.39 mg. metallic lead on 319 gram body weight, a lower value for the testicles than for the liver could have been expected. The obtained values indicate a concentration of lead in the testicles.

CHAPTER VI.

The Effect of Thorium Trypan Blue and Castration on Induced Cancer in White Mice by Means of Painting with Tar, Dibenzpyrene and Methylcholanthrene.

1. The occuroence of tumours.

In this investigation, as stated on page 121, the aim was, among others, to examine the effect of the treatment with thorium trypan blue and castration on mouse-cancer induced with dibenzpyrene. Because of the lack of dibenzpyrene, tar had to be used on some few mice (series V: 237—317). For series VI during the last few weeks of the treatment and for series VIII during the whole period, methylcholanthrene was used. As it appeared that the treatment with lead trypan blue seemed to have an effect on the males but not on the females, one then wished through treating with thorium trypan blue to try the effect on both normal and castrated animals, by means of which one could also see the effect of castration. Treatment with thorium trypan blue began first when the animals had developed tumours. Accordingly, prior to this time it is possible, as regards the time of the tumour's origin and the frequency of tumour formation, to compare the conditions in all the normal and castrated animals in the material.

When comparing males and females, one must partly take into con-

Table 22. *The number of tumours occurring at different time periods after the beginning of the painting in relation to the number of painted animals.* Series IV, V, VI and VIII.

	Weeks	−6	−8	−10	−12	−14	−16	−18	−20	−22	−24	−26	−28	−30	−32	<32	S:a
Castrated	♂ Series V, VI, VIII obs.*	1	—	31	24	56	40	7	9	2	—	—	1	3	—	6	180
	%**	0.5	0	15.4	11.9	27.9	19	3.5	4.5	1.0	—	—	0.5	1.5	—	3	89.6
	♀ Series IV, V, VI obs.	—	6	9	25	26	13	5	12	—	3	3	4	1	4	5	116
	%	—	6.5	9.8	27.2	28.3	7.3	2.8	6.7	—	1.7	1.7	2.2	0.6	2.2	2.8	64.8
	♂ + ♀	1	6	40	49	82	53	12	21	2	3	3	5	4	3	11	296
	%	0.3	1.6	10.5	12.9	21.6	13.9	3.2	5.5	0.5	0.8	0.8	1.3	1.1	0.8	2.9	77.9
Normal	♂ Series V, VI, VIII	—	2	28	19	43	19	—	5	—	1	—	—	—	—	—	117
	%	—	1.5	21.2	14.4	32.6	14.4	—	3.8	—	0.8	—	—	—	—	—	88.6
	♀ Serie IV, V (partly) and VI	1	2	11	11	20	10	4	5	3	8	2	—	2	—	3	82
	%	0.8	1.5	8.3	8.3	15.2	7.6	3.0	3.8	2.3	6.1	1.5	—	1.5	—	2.3	82
	The above ♂ + ♀ Series IV, V, VI, VIII	1	4	39	30	63	27	4	10	3	9	2	—	2	—	3	197
	%	0.4	1.5	14.8	11.4	23.9	10.2	1.5	3.8	1.1	3.4	0.8	—	0.8	—	1.1	74.6
	♀ Series V: No. 237—317 tar-painted	—	—	—	9	13	—	12	20	13	4	6	—	—	—	—	77
	%	—	—	—	11.1	16.0	—	14.8	24.7	16.0	4.9	7.4	—	—	—	—	62.1

* obs. = observed number. ** % = tumours in per cent of the number painted animals.

sideration the females in series V which were tar-painted all the time, and which developed tumours later than the dibenzpyrene-treated females (see page 128). Consideration must also be taken to the fact that the material of female mice in series IV is equally distributed among normal and castrated females, but with a considerably later origin of tumours than in the following series.

The tumour frequency in the males which have been castrated before they were painted is equally as high as in the normal males, the difference being 1.0 ± 3.5 per cent. The tumour frequency in the castrated females is also equally as high as in the normal females, the difference is 2.7 ± 5.5 per cent. Castration seems to have no effect on the tendency to develop tumours after tar-painting. The frequency is true of an observation period of up to one year. *If the frequency in* (castrated and normal) *female mice is compared to* (castrated and normal) *male mice the tumour frequency is 99.3 and 89.2 per cent respectively, the difference is 10.1 ± 1.8 per cent and thus statistically significant* (the females in series IV and no. 237—317 in series V are not included in this calculation). As the female mice in this material, as well as those in that dealt with in the preceeding chapter, have a higher tumour frequency than the male mice, one ventures to assume that *female mice more easily develop skin tumours through a carcinogenic influence than the males.* It is possible that this difference is because of the difference between the hormonal conditions in female and male mice. That castration has no effect contradicts, however, to some degree this assumption.

When studying the distribution of the occurence of tumours during different periods it seems to be approximately equal in the female mice. In the male mice, on the other hand, a tendency to a later development of tumours in the castrated mice is found as compared to the normal ones. This difference becomes apparent when calculating that time which has, on an average, elapsed before the onset of the tumour. This is for

191 castrated males	91.9 ± 2.3	days
121 normal males	81.1 ± 1.8	»
114 castrated females	106.2 ± 4.9	»
132 normal females	106.8 ± 5.7	»

It appears from the above figures that *castration is of no influence on the time required to produce tumours in female mice.*

Contrary to this, *in the male mice a difference exists which is 9.76 ± 2.93 per cent and thus statistically significant.*

In order to make a more complete comparison, I have also calculated the median and quartiles as to the origin of tumours in the different groups. The figures obtained are found in table 23. They show, on the whole good agreement, apart from the castrated males for which the figures are lower.

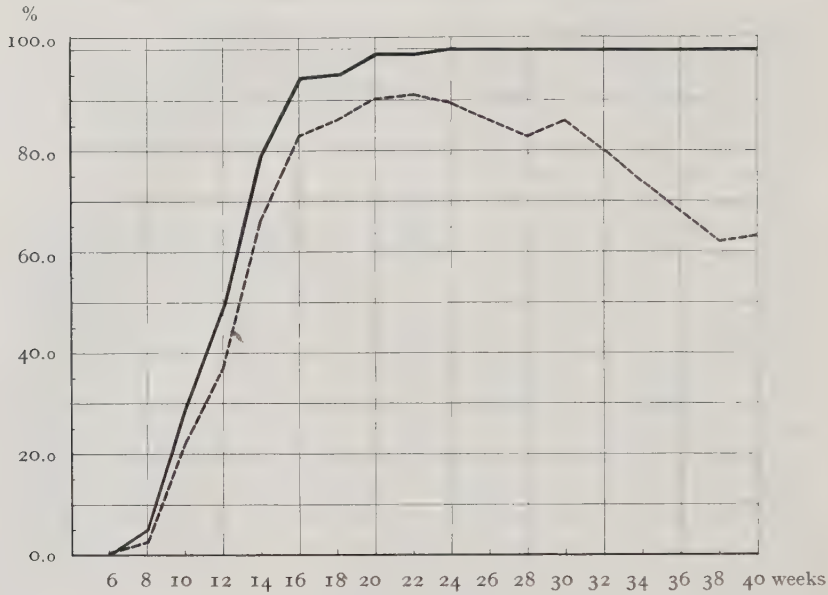


Diagram 4. The frequency of tumourbearing animals in per cent of the number surviving animals at different periods after the beginning of the painting. Series V, VI, VIII.

——— = normal males - - - - - - - - = castrated males

Table 23. *The time in weeks before the development of the tumours after the beginning of the painting.*

	Number	Lower quartile	Median	Upper quartile
	Castrated animals			
♂ Series V, VI and VIII	180	11.1	13.2	15.2
♀ Series IV, V and VI	116	11.1	13.4	18.6
♂ + ♀	296	11.1	13.3	15.7
	Normal animals			
♂ Series V, VI and VIII	117	10.0	12.5	13.8
♀ Series IV, V (in part) and VI	82	11.2	13.7	19.3
♂ + ♀ Series IV, V, VI and VIII	197	10.4	12.8	14.9
♀ Series V: No. 236—317 (tar-painted)	77	13.6	18.5	20,7

From table 24 the number of surviving mice in relation to the number of painted mice appears. It is seen here that the castrated male and female mice have a certain tendency to live longer than the normal males and females. In series VIII which comprises only male mice, a consi-

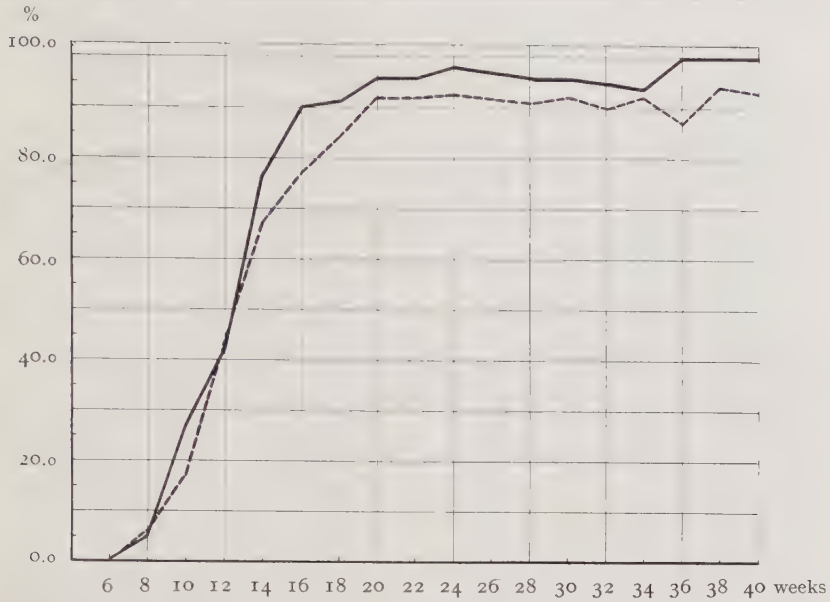


Diagram 5. The frequency of tumourbearing animals in per cent of the number surviving animals at different periods after the beginning of the painting. Series V, VI.

——— = normal females - - - - - - - - = castrated females

derably shortened life is found (see page 173). This is an important source of error due to the different number of castrated and normal mice in other series. Furthermore, series IV has a low tendency to develop tumours and a long latent period before developing the tumour. If a comparison is made between castrated and non-castrated mice in series V and VI (apart from no. 237—317 in series V), *a certain tendency to a lower mortality is found in the castrated mice*, which fact, however, does not manifest itself until the higher ages are reached when the tumours generally have had time to form. This difference is statistically probable at 26, 34, 36, and 38 weeks, and significant at 30 and 40 weeks. If a comparison is made between only castrated and normal male mice, the same tendency is found though not so pronounced. In female mice no significant figures as to the differences are obtained.

In this investigation it occurs that some of the mice develop tumours very late, or not at all. In the table only periods of up to 40 weeks after the beginning of painting have been considered as has been previously mentioned. In rare cases it occurs that the mice developed tumours even later. In order to get an idea as to whether castration possibly influences the time for the origin of tumours, a diagram is given showing the number of tumours in relation to the number of surviving mice at different periods (diagram 4, 5).

Table 24 a. *Number of surviving animals in per cent of painted animals.* Series IV, V, VI and VIII.

		0	-2	-4	6	—8	—10	—12	-14	-16	—18	20	—22	—24	—26	—28	-30	—32	—34	36	38	40
Castr. ♂	obs.	201	199	199	199	199	199	197	197	195	185	180	170	145	101	83	79	54	43	34	29	21
	%	100.0	99.0	99.0	99.0	99.0	99.0	98.0	98.0	97.0	92.0	89.6	84.6	72.0	50.0	41.3	39.3	26.9	21.4	16.9	14.4	10.4
Norm. ♂	obs.	132	129	127	126	126	125	125	123	118	111	107	95	67	53	28	21	17	12	6	3	1
	%	100.0	97.7	97.7	95.5	95.5	94.7	94.7	93.2	89.4	84.1	81.1	72.0	50.8	40.2	21.2	15.9	12.9	9.1	4.5	2.3	0.8
Castr. ♂ except. series VIII	obs.	124	122	122	122	122	122	120	120	119	118	117	114	97	89	78	78	54	43	34	29	21
	%	100.0	98.4	98.4	98.4	98.4	98.4	96.8	96.8	96.0	95.2	94.4	91.9	78.2	71.8	62.9	62.9	43.5	34.7	27.4	23.4	16.9
Norm. ♂ except. series VIII	obs.	58	58	58	58	58	58	58	57	56	56	54	52	38	31	26	20	17	12	6	3	1
	%	100.0	100.0	100.0	100.0	100.0	100.0	100.0	98.3	96.6	96.6	93.1	89.7	65.5	53.4	44.8	34.5	29.3	20.7	10.3	5.2	1.7
Castr. ♀	obs.	179	165	153	142	134	133	130	125	122	119	115	105	92	87	80	73	61	57	36	30	27
	%	100.0	92.2	85.5	79.3	74.9	74.3	72.6	69.8	68.2	66.5	64.2	58.7	51.4	48.6	44.7	40.8	34.8	31.8	20.1	16.8	15.1
Norm. ♀	obs.	132	125	117	111	109	107	101	96	93	89	86	79	67	57	48	44	37	32	26	22	12
	%	100.0	94.7	88.6	84.1	82.6	81.1	76.5	72.7	70.5	67.4	65.2	59.8	50.8	43.2	36.4	33.3	28.0	24.2	19.7	16.7	9.1
Castr. ♀ except series IV	obs.	93	93	93	90	90	90	90	88	88	86	85	78	67	64	56	49	40	38	23	18	15
	%	100.0	100.0	100.0	96.8	96.8	96.8	96.8	94.6	94.6	92.5	91.4	83.9	72.0	68.8	60.2	52.7	43.0	40.9	24.7	19.4	16.1
Norm. ♀ except series IV	obs.	59	59	59	59	59	59	59	58	58	57	54	50	43	35	28	26	21	16	11	10	3
	%	100.0	100.0	100.0	100.0	100.0	100.0	100.0	98.3	98.3	96.6	91.5	84.7	72.9	59.3	47.5	44.1	35.6	27.1	18.6	16.9	5.1
Castr. ♂ + ♀ except series IV, VIII	obs.	217	215	215	212	212	212	210	208	207	204	202	192	164	153	134	127	94	81	57	47	36
	%	100.0	99.1	99.1	97.7	97.7	97.7	96.8	95.9	95.4	94.0	93.1	88.5	75.6	70.5	61.8	58.5	43.3	37.3	26.3	21.7	16.6
Norm. ♂ + ♀ except series IV, VIII	obs.	117	117	117	117	117	117	117	115	114	113	108	102	81	66	54	46	38	28	17	13	4
	%	100.0	100.0	100.0	100.0	100.0	100.0	100.0	98.3	97.4	96.6	92.3	87.2	69.2	56.4	46.2	39.3	32.5	23.9	14.5	11.1	3.4

Obs. = observed number. Castr. = castrated. Norm. = not castrated. % = per cent of painted animals.

Table 24 b. *Number of surviving animals in per cent of painted animals.* Series IV.

	0	2	—4	6	8	—10	12	14	—16	—18	—20	—22	—24	—26	—28	—30	—32	—34	—36	—38	—40
Castrated surviving females	86	72	60	52	49	43	40	37	34	33	30	27	25	25	24	24	21	19	13	12	12
% of painted animals	100.0	83,7	69.8	60.5	51.2	50.0	46.5	43.0	39.5	38.4	34.9	31.4	29.1	29.1	27.9	27.9	24.4	22.1	15.1	14.0	14.0
Normal surviving females	73	66	59	52	50	48	42	38	35	32	32	29	24	22	20	18	16	16	15	12	11
% of painted animals	100.0	90.4	80.8	71.2	68.5	65.8	57.5	52.1	47.9	43.8	43.8	39.7	32.9	30.1	27.4	24.7	21.9	21.9	20.5	16.4	15.1

Table 24 c. *Number of surviving animals in per cent of painted animals.* Series VIII.

	0	—2	—4	—6	—8	—10	—12	—14	—16	—18	—20	—22	—24	—26	—28	—30
Surviving ♂	77	77	77	77	77	77	77	77	76	67	63	56	48	12	5	1
% of painted animals	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	98.7	87.0	81.8	72,7	62.3	15.6	6.5	1.3
Surviving normal ♂	74	71	70	69	69	67	67	66	62	53	53	43	29	22	2	1
% of painted animals	100.0	95.9	94.6	93.2	93.2	90.5	90.5	89.2	83.8	71.6	71.6	58.1	39.2	29.7	2.7	1.4

Table 25. *Number of animals which died spontaneously with tumour in relation to the number of painted animals at different periods as counted from the development of the tumours (= at different tumour-ages).* Series IV, V (no. 237—317 excl.), VI, VIII.

	Weeks	—2	—4	—6	—8	—10	—12	—14	—16	—18	—20	—22	—24	—26	>26	Total
Castrated males	non-tr.	—	1	2	3	11	13	10	11	11	7	2	3	2	—	76
	%	—	1.0	2.1	3.1	11.3	13.4	10.3	11.3	11.3	7.2	2.1	3.1	2.1	—	78.4
	tr.	2	—	2	7	9	12	11	9	5	8	6	3	3	3	80
	%	1.9	—	1.9	6.7	8.7	11.5	10.6	8.7	4.8	7.7	5.8	2.9	2.9	2.9	76.9
Normal males	non-tr.	—	2	1	3	6	2	15	12	7	3	5	9	1	—	66
	%	—	2.1	1.0	3.1	6.3	2.1	15.6	12.5	7.3	3.1	5.2	9.4	1.0	—	68.8
	tr.	1	1	1	1	6	10	9	3	—	—	—	—	—	—	32
	%	2.8	2.8	2.8	2.8	16.7	27.8	25.0	8.3	—	—	—	—	—	—	88.9
Castrated females	non-tr.	—	1	3	2	5	3	10	6	2	1	4	3	3	—	43
	%	—	1.1	3.2	2.1	5.3	3.2	10.6	6.4	2.1	1.1	4.3	3.2	3.2	—	45.7
	tr.	—	2	—	—	—	4	6	6	4	6	7	4	1	2	42
	%	—	2.4	—	—	—	4.7	7.1	7.1	4.7	7.1	8.2	4.7	1.2	2.4	49.4
Normal females	non-tr.	—	2	5	2	5	6	6	11	7	6	3	6	1	2	62
	%	—	1.5	3.8	1.5	3.8	4.5	4 5	8.3	5.3	4.5	2.3	4.5	0.7	1.5	47.0

Non tr. = non-treated. Tr. = treated. % = per cent of number of painted animals.

In addition to table 25 a comparison is given between castrated animals (males and females) and non-castrated (males and females) animals (except Series IV and VIII).

24 w.	6.4 ± 5.2	not proved	34 w.	13.4 ± 5.1	probable
26 w.	14.1 ± 5.5	probable	36 w.	11.8 ± 4.5	»
28 w.	13.6 ± 5.6	not proved	38 w.	10.6 ± 4.0	»
30 w.	19.2 ± 5.6	significant	40 w.	13.2 ± 3.0	significant
32 w.	10.8 ± 5.5	not proved			

Comparison between castrated and normal males (except Series VIII).

24 w.	11.7 ± 7.3	not proved	34 w.	14.0 ± 6.8	not proved
26 w.	18.4 ± 7.7	» »	36 w.	10.1 ± 5.7	probable
28 w.	18.1 ± 7.8	» »	38 w.	18.2 ± 4.8	significant
30 w.	28.4 ± 7.6	significant	40 w.	15.2 ± 3.8	»
32 w.	14.2 ± 7.5	not proved			

From the above diagram it appears that the tumour formation is similar in castrated and normal female mice with a slight tendency to inhibition in the castrated females. Statistical calculation yields no proved difference. On the other hand, a clear inhibition in the male mice is found, the tumour formation being retarded so that a significant number of mice do not develop tumours during the 40 weeks considered

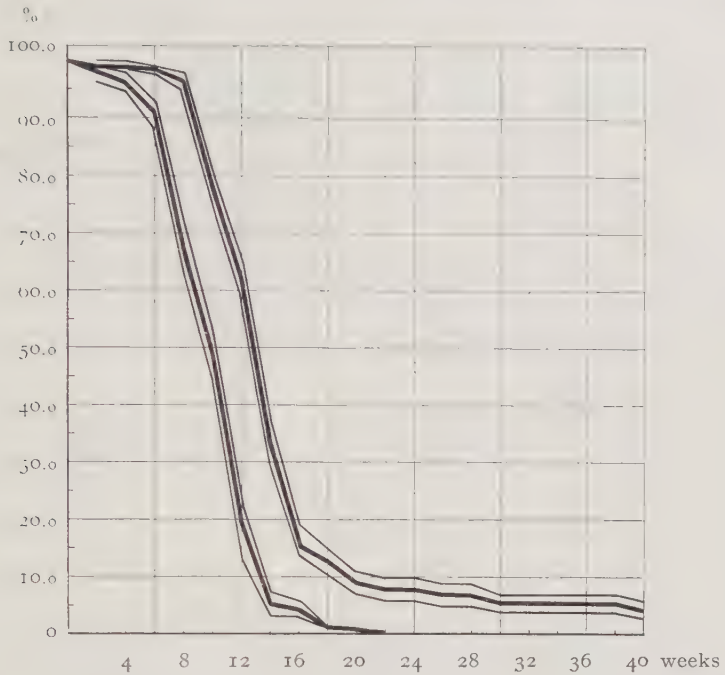


Diagram 6. The number surviving animals without tumour at different periods in relation to the number painted animals. The figures are given in per cent with one time standard error. The upper curve = castrated males. The lower curve = non-castrated males. Series V, VI, VIII.

in this calculation. If the individual figures for the castrated and normal male mice are calculated, it is found that the normal males possess a greater tendency to develop tumours; whereby the difference from the 20th to 40th week becomes statistically significant. The result can be seen in the diagram below which shows the curves for the castrated and normal male mice with their standard error.

A table showing the number of mice which died spontaneously of malignant tumours at different periods in relation to the number of painted mice is given.

As appears from the table no significant difference in mortality is found.

Summarizing, it appears from the above mentioned that *castration delays the occurrence of tumours in male mice but has no effect on the females in this respect.*

As regards the time after the origin of the tumours the subsequent course may be affected by the treatment with thorium trypan blue and

castration. In all cases where it is possible, a comparison will be made in the proceeding between the thoriumtrypan blue-treated and non-treated mice, and between castrated and normal mice.

When comparing different groups of test animals it is, as mentioned previously, necessary to take into consideration the deviations in series IV, no. 237—317 in series V and in series VIII. In the information given below as to the average tumour-age in mice which died spontaneously, no. 237—317 in series V and series VIII are therefore accounted for individually; then one can easily read the extent of the influence of the treatment with thorium trypan blue and castration on the duration of the life of the mice after the origin of the tumour.

The average tumour-age in mice that died spontaneously:

Series IV, V, VI.		
63 normal, non-treated females	102.9 ± 5.4	days
43 castrated, non-treated females	99.8 ± 6.3	»
42 castrated, treated females	120.0 ± 6.3	»
Series V (No. 236—317).		
30 normal non-treated females	126.3 ± 7.0	»
27 normal, treated females	131.5 ± 6.4	»
Series V, VI.		
48 normal, non-treated males	118.0 ± 5.0	»
44 castrated, non-treated males	112.8 ± 4.4	»
46 castrated, treated males	119.9 ± 6.3	»
Series VIII.		
18 normal, non-treated males	73.8 ± 6.9	»
32 normal, treated males	75.1 ± 4.1	»
32 castrated, non-treated males	76.4 ± 4.1	»
33 castrated, treated males	71.2 ± 3.9	»

As can be seen from the above figures, the greatest difference is found between thorium trypan blue-treated and non-treated, castrated female mice. However, the difference is 20.2 ± 8.9 and thus not statistically proved, but it is, however, probable. A more comprehensive material is required to determine this question. A slight difference seems to exist between thorium trypan blue-treated, castrated males and non-treated, castrated males. A comparison between thorium trypan blue-treated, castrated male and female mice, on the one side, and non-treated, castrated male and femalae mice on the other side, shows a difference of 13.0 ± 5.9, so the difference is neither proved here. Thus, neither the treatment with thorium trypan blue nor castration seems to influence the duration of their lives after the origin of the tumour, or more cautiously expressed, the influence of these factors is at any rate so slight that it cannot be proved in this material.

Table 26. *The animals' weight at different periods in weeks after appearance of the tumours.*

Weeks	Males			Females			Males			Females		
	n	$M \pm \varepsilon (M)$	σ	n	$M \pm \varepsilon (M)$	σ	n	$M \pm \varepsilon (M)$	σ	n	$M \pm \varepsilon (M)$	σ
	Normal non-treated						Castrated non-treated					
1	72	30.17 ± 0.43	3.62	113	26.49 ± 0.25	2.69	69	30.91 ± 0.34	4.47	36	28.76 ± 0.56	3.36
3	72	29.65 ± 0.43	3.62	111	26.22 ± 0.26	2.78	68	30.48 ± 0.50	4.14	36	28.90 ± 0.55	3.30
5	71	30.20 ± 0.45	3.82	108	26.43 ± 0.31	3.19	68	29.72 ± 0.51	4.22	36	28.60 ± 0.48	2.90
7	70	30.55 ± 0.48	4.01	102	26.82 ± 0.30	3.05	65	30.07 ± 0.48	3.88	36	29.29 ± 0.57	3.40
9	65	30.52 ± 0.52	4.16	99	26.67 ± 0.34	3.40	62	29.85 ± 0.43	3.37	34	30.16 ± 0.57	3.33
	Normal, treated						Castrated, treated					
1	33	29.65 ± 0.30	1.75	38	27.80 ± 0.41	2.50	71	30.06 ± 0.37	3.13	41	28.95 ± 0.63	4.05
3	32	29.33 ± 0.39	2.20	38	28.11 ± 0.48	2.96	69	30.07 ± 0.39	3.21	40	28.74 ± 0.61	3.86
5	30	28.85 ± 0.42	2.32	38	27.74 ± 0.50	3.05	68	30.13 ± 0.40	3.33	40	29.10 ± 0.67	4.24
7	27	29.09 ± 0.50	2.61	38	27.45 ± 0.49	2.99	64	30.36 ± 0.44	3.49	40	29.68 ± 0.68	4.28
9	26	29.06 ± 0.46	2.35	36	27.51 ± 0.46	2.78	61	30.76 ± 0.44	3.44	40	29.93 ± 0.71	4.50

Table 27. *Difference in weight between males and females at different periods in weeks after appearance of the tumours.*

Weeks	Normal, treated males-females	Castrated, treated males-females	Normal non-treated males-females	Castrated non-treated males-females
1	1.85 ± 0.51	1.11 ± 0.73	3.68 ± 0.50	2.15 ± 0.78
3	1.22 ± 0.62	1.33 ± 0.72	3.43 ± 0.50	1.58 ± 0.74
5	1.11 ± 0.65	1.03 ± 0.78	3.77 ± 0.55	1.12 ± 0.70
7	1.64 ± 0.70	0.68 ± 0.81	3.73 ± 0.57	0.78 ± 0.75
9	1.55 ± 0.65	0.83 ± 0.84	3.91 ± 0.62	0.31 ± 0.71

As has been shown previously, treatment with thorium trypan blue yielded no provable shortening of the length of life of the tumour-bearing animals as reckoned from the beginning of the experiment. In order to see wether the body-weight had altered due to the treatment as a symptom of the treatments effect on the general condition of the animals, the body-weight with standard error including the curves have been given in the table below. In this experiment no positive effect of castration on the body-weight could be found. The treatment with thorium trypan blue had a certain but not provable decreasing effect on the body weight at the end of the experiment perhaps somewhat more pronounced in the females.

Table 28. *The animals' weight at different periods in weeks before appearnce of the tumours.*

Weeks	Males			Females			Males			Females		
	n	$M \pm \varepsilon (M)$	σ	n	$M \pm \varepsilon (M)$	σ	n	$M \pm \varepsilon (M)$	σ	n	$M \pm \varepsilon (M)$	σ
	Normal non-treated						Castrated non-treated					
0	73	26.93 ± 0.44	3.78	114	24.07 ± 0.25	2.66	70	28.50 ± 0.40	3.38	36	27.44 ± 0.46	2.75
2	73	28.22 ± 0.39	3.33	114	25.30 ± 0.26	2.76	70	29.62 ± 0.46	3.84	36	28.93 ± 0.45	2.68
4	73	28.57 ± 0.37	3.19	114	25.47 ± 0.25	2.62	70	30.22 ± 0.51	4.24	36	29.19 ± 0.51	3.06
6	68	28.65 ± 0.46	3.83	113	25.48 ± 0.22	2.34	70	30.59 ± 0.48	3.98	36	29.19 ± 0.51	3.06
8	47	28.78 ± 0.34	2.33	103	25.67 ± 0.24	2.45	69	30.64 ± 0.46	3.85	35	28.84 ± 0.52	3.06
10	31	29.90 ± 0.44	2.43	86	25.06 ± 0.27	2.50	49	30.88 ± 0.53	3.73	19	29.45 ± 0.83	3.60
12	15	29.77 ± 0.51	1.99	67	26.55 ± 0.28	2.27	26	30.56 ± 0.78	3.99	11	29.09 ± 1.18	3.90
14	6	29.33	—	56	25.68 ± 0.30	2.27	17	30.47 ± 1.13	4.65	8	28.31	—
16	5	29.30	—	49	25.78 ± 0.31	2.19	11	30.77 ± 1.35	4.49	5	29.50	—
	Normal, treated						Castrated, treated					
0	33	26.95 ± 0.25	1.46	38	26.59 ± 0.45	2.78	71	27.48 ± 0.69	5.83	41	27.45 ± 0.55	3.53
2	33	28.15 ± 0.29	1.67	38	26.86 ± 0.44	2.70	71	29.07 ± 0.41	3.48	41	28.61 ± 0.58	3.74
4	33	28.33 ± 0.36	2.08	38	26.36 ± 0.38	2.30	71	29.90 ± 0.45	3.80	41	29.40 ± 0.66	4.23
6	32	28.61 ± 0.39	2.18	38	27.04 ± 0.42	2.56	70	29.68 ± 0.48	4.01	41	29.16 ± 0.64	4.13
8	26	28.37 ± 0.37	1.87	38	27.30 ± 0.39	2.41	64	30.25 ± 0.46	3.68	41	29.12 ± 0.71	4.56
10	21	30.40 ± 0.25	1.17	36	27.96 ± 0.40	2.40	52	31.09 ± 0.45	3.25	31	29.50 ± 0.81	4.50
12	3	29.67	—	34	28.28 ± 0.44	2.56	24	29.46 ± 0.65	3.20	16	29.34 ± 1.03	4.11
14	3	29.00	—	29	28.12 ± 0.70	2.64	10	28.85 ± 1.06	3.36	9	27.39	—
16	1	29.00	—	21	27.60 ± 0.49	2.23	5	26.80	—	6	27.33	—

Table 29. *Difference in weight between males and females at different periods in weeks before appearance of the tumours.*

Weeks	Normal, treated males-females	Castrated, treated males-females	Normal non-treated males-females	Castrated non-treated males-females
0	0.36 ± 0.51	0.03 ± 0.88	2.86 ± 0.51	1.06 ± 0.61
2	1.29 ± 0.53	0.46 ± 0.71	2.92 ± 0.47	0.69 ± 0.64
4	1.97 ± 0.52	0.50 ± 0.80	3.10 ± 0.45	1.03 ± 0.72
6	1.57 ± 0.57	0.52 ± 0.80	3.17 ± 0.51	1.40 ± 0.70
8	1.07 ± 0.55	1.13 ± 0.85	3.11 ± 0.42	1.80 ± 0.69
10	2.44 ± 0.47	1.59 ± 0.93	3.84 ± 0.52	1.43 ± 0.98
12	—	0.12 ± 1.22	3.22 ± 0.58	1.47 ± 1.41

The given thorium trypan blue doses.

When treating with thorium trypan blue the animals were given subcutaneous injections three times a week corresponding to 2 per cent of the body weight. Calculated in metallic thorium the given doses were as follows

Diagrams 7—10. *The animals weight at different periods before and after the occurrence of the tumours.*

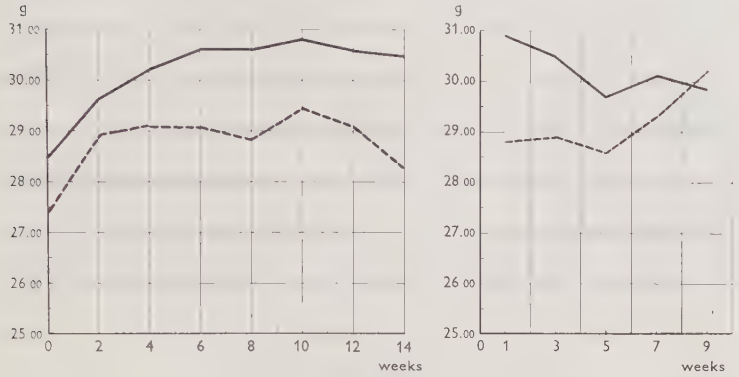


Diagram 7.

——— = castrated non-treated males.
- - - - - - - - - = castrated non-treated females.

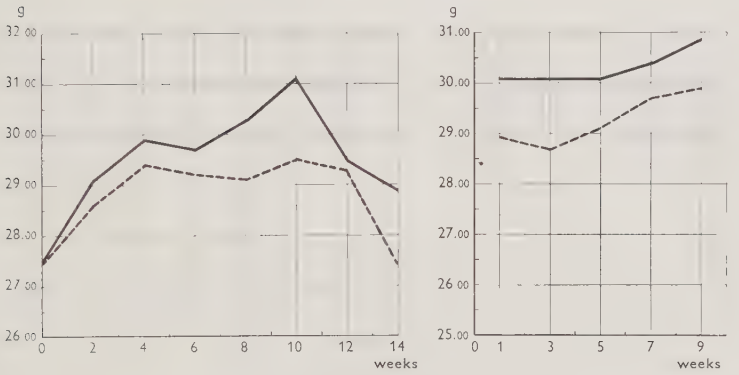


Diagram 8.

——— = castrated treated males.
- - - - - - - - - = castrated treated females.

Series V, VI	castrated males	0,31±0.01 mg. (n = 58)
	castrated females	0.30±0.02 mg. (n = 47)
	normal females	0.30±0.01 mg. (n = 37)
Series VIII	castrated males	0.14±0.01 mg. (n = 32)
	normal males	0.16±0.01 mg. (n = 22)

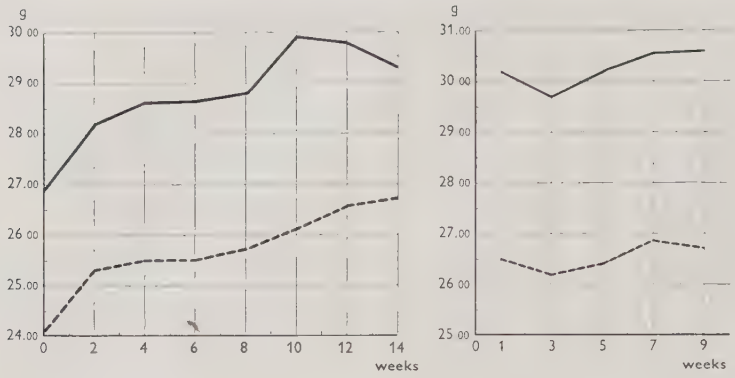


Diagram 9.

——— = normal non-treated males.
- - - - - - - - - = normal non-treated females.

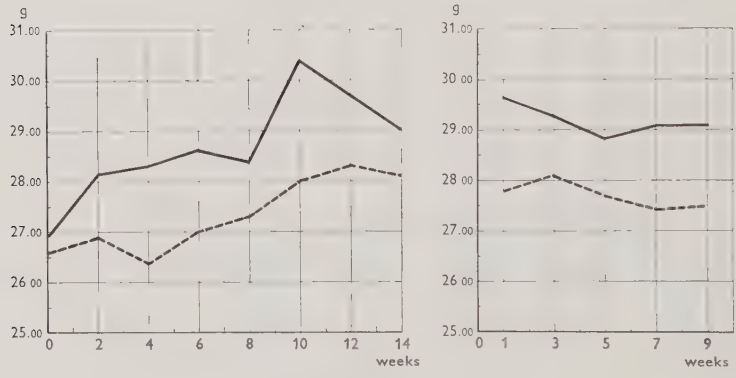


Diagram 10.

——— = normal treated males.
- - - - - - - - - = normal treated females.

Influence on the weight of internal organs.

On the whole no influence of castration or treatment with thorium trypan blue on the weight of internal organs could be proved. As it is known that hormone treatment of castrated animals will increase the compensatoric hypertrophy of the kidney left in mice (Wrete) it is of some interest to see whether the weight of the kidneys is altered in castrated mice. The fixed decapsulated kidneys were weight at the

section of the animals. The results are as follows difference between non-castrated and castrated males — 10.5 ± 3.78 mg. and thus statistically probable, difference between non-castrated (males and females) and castrated (males and females) = 13.8 ± 4.4 mg. and thus statistically significant,
difference between normal treated (males and females) and normal non-treated (males and females) = 16.2 ± 4.9 mg. and thus statistically significant.

As the animals used for these calculations are of the same sizes in the different groups it is probable, that castration decreased the weight of the kidneys. Contrarily treatment with thorium trypan blue increases the kidneys' weight. In the material of lead trypan blue-treated animals no differences could be shown.

The rate of tumour growth.

When comparing the rate of growth of the tumours in mg. per day no provable differences were found in the various test groups. This may partly depend on the fact that the animals belonged to different strains. If in consideration out of this the animals in series V and VI are used and compared the thorium trypan blue-treated (castrated males and females and normal females) with non-treated (castrated males and females and normal females) the difference becomes 8.2 ± 4.5 mg. and thus not proved. The treated animals have longer tumour-age than the non-treated animals, the difference is 12.8 ± 4.8 days and thus statistically probable. But no difference could be found between the castrated and the normal animals.

2. Metastasizing of the tumours.

As in the preceeding chapter, it is also of great importance when treating the mice with thorium trypan blue and castration to consider the distribution of the tumours as being a significant symptom of malignity. Table 31 gives a survey of metastasizing of the tumours to the regional lymph nodes and internal organs. (For further details is referred to the table deposited at the University Library at Uppsala.) When judging the results, one must distinguish between the effect of the treatment with thorium trypan blue and the influence of castration on the metastasizing. Here, a comparison of the metastasizing frequency will be made between the non-treated (normal and castrated) and treated (normal and castrated), in order to establish the influence of the treatment with thorium trypan blue. Furthermore, the metastasizing frequency is compared between normal (non-teated and treated), and castrated (non-treated and treated) in order to observe the effect of castration. If the

Tables 30 a—l. *Tables of the incidence of metastases at varying tumour-age.*

Table 30 a. Series V, VI. Males. Castrated, non-treated.

Days	0—30	31—60	61—90	91—120	121—150	>150	Total
benign. tumour	1	—	—	—	—	—	1
tumour without metastasis	—	2	1	6	5	5	19
tumour with metastases of the lymph nodes only	—	—	1	4	1	4	10
tumour with metastases of the lungs only	—	—	—	2	2	—	4
tumour with metastases in two sites	—	—	3	6	5	3	17
tumour with metastases in more than two sites	—	—	—	1	1	—	2
total	1	2	5	19	14	12	53

Table 30 b. Series V, VI. Males. Normal, non-treated.

Days	0—30	31—60	61—90	91—120	121—150	>150	Total
benign. tumour	—	—	—	—	—	—	—
tumour without metastasis	1	1	1	4	4	7	18
tumour with metastases of the lymph nodes only	—	—	2	2	2	5	11
tumour with metastases of the lungs only	—	—	—	3	2	3	8
tumour with metastases in two sites	—	—	2	4	1	2	9
tumour with metastases in more than two sites	—	—	2	1	1	—	4
total	1	1	7	14	10	17	50

Table 30 c. Series V, VI. Males. Castrated, treated with thorium trypan blue.

Days	0—30	31—60	61—90	91—120	121—150	>150	Total
benign. tumour	—	—	—	—	—	—	—
tumour without metastasis	1	1	4	3	4	8	21
tumour with metastases of the lymph nodes only	—	—	1	2	3	1	7
tumour with metastases of the lungs only	—	—	—	—	2	3	5
tumour with metastases in two sites	—	1	1	5	6	3	16
tumour with metastases in more than two sites	—	—	1	1	3	3	8
total	1	2	7	11	18	18	57

Table 30 d. Series VIII. Males. Normals, non-treated.

Days	0— 30	31— 60	61— 90	91— 120	121— 150	Total
benign. tumour	—	—	—	—	—	—
tumour without metastasis	2	3	6	5	1	17
tumour with metastases of the lymph nodes only	—	—	—	1	—	1
tumour with metastases of the lungs only	—	—	—	—	—	—
tumour with metastases in two sites	—	—	—	1	—	1
tumour with metastases in more than two sites	—	—	—	—	—	—
total	2	3	6	7	1	19

Table 30 e. Series VIII. Males. Castrated, treated with thorium trypan blue.

Days	0— 30	31— 60	61— 90	91— 120	121— 150	Total
benign. tumour	—	—	1	—	—	1
tumour without metastasis	1	9	14	3	—	27
tumour with metastases of the lymph nodes only	—	—	3	2	—	5
tumour with metastases of the lungs only	—	—	—	—	—	—
tumour with metastases in two sites	—	—	1	—	—	1
tumour with metastases in more than two sites	—	—	—	—	—	—
total	1	9	19	5	—	34

Table 30 f. Series VIII. Males. Castrated, non-treated.

Days	0— 30	31— 60	61— 90	91— 120	121 150	Total
benign. tumour	—	—	—	—	—	—
tumour without metastasis	1	4	8	5	—	18
tumour with metastases of the lymph nodes only	—	—	4	4	—	8
tumour with metastases of the lungs only	—	1	2	—	—	3
tumour with metastases in two sites	—	—	—	—	—	—
tumour with metastases in more than two sites	—	—	—	—	—	—
total	1	5	14	9	—	29

Table 30 g. Series VIII. Males. Normals, treated with thorium trypan blue.

Days	0—30	31—60	61—90	91—120	121—150	Total
benign. tumour	1	—	—	—	—	1
tumour without metastasis	3	2	15	4	—	24
tumour with metastases of the lymph nodes only	—	—	—	4	—	4
tumour with metastases of the lungs only	—	—	—	—	—	—
tumour with metastases in two sites	—	—	3	1	—	4
tumour with metastases in more than two sites	—	—	—	—	—	—
total	4	2	18	9	—	33

Table 30 h. Series IV, V, VI. Females. Castrated, non-treated.

Days	0—30	31—60	61—90	91—120	121—150	>150	Total
benign. tumour	1	—	—	—	—	—	1
tumour without metastasis	1	4	6	3	2	6	27
tumour with metastases of the lymph nodes only	—	1	1	2	1	2	7
tumour with metastases of the lungs only	—	—	1	—	1	3	5
tumour with metastases in two sites	—	1	3	4	2	3	13
tumour with metastases in more than two sites	—	—	1	2	1	1	5
total	2	6	12	11	7	15	53

Table 30 i. Series IV, V, VI. Females. Castrated, treated with thorium trypan blue.

Days	0—30	31—60	61—90	91—120	121—150	>150	Total
benign. tumour	—	1	—	2	—	1	4
tumour without metastasis	2	—	4	3	5	13	27
tumour with metastases of the lymph nodes only	—	—	1	—	2	—	3
tumour with metastases of the lungs only	—	—	1	4	1[1]	1	6 (+1)
tumour with metastases in two sites	—	—	2	3	7	3	15
tumour with metastases in more than two sites	—	—	—	—	2	7	9
total	2	1	8	12	17	25	65

[1] Metastasis of the suprarenal gland.

Table 30 j. Series IV, V (except no 237—317), VI. Females. Normal, non-treated.

Days	0—30	31—60	61—90	91—120	121—150	>150	Total
benign. tumour	3	1	—	—	—	—	4
tumour without metastasis	2	3	2	7	4	5	23
tumour with metastases of the lymph nodes only	1	1	3	6	2	2	15
tumour with metastases of the lungs only	—	1	1	2	1	4	9
tumour with metastases in two sites	—	2	6	4	5	4	21
tumour with metastases in more than two sites	—	—	1	1	—	2	4
total	6	8	13	20	12	17	76

Table 30 k. Series V, no 237—317. Females. Normal, non treated.

Days	0—30	31—60	61—90	91—120	121—150	>150	Total
benign. tumour		—	—	—	—	—	—
tumour without metastasis	—	2	—	—	—	1	3
tumour with metastases of the lymph nodes only	—	—	2	3	1	4	10
tumour with metastases of the lungs only	—	—	1	—	—	5	6
tumour with metastases in two sites	—	—	1	5	5	5	13
tumour with metastases in more than two sites	—	—	1	—	3	1	5
total	—	2	5	5	9	16	37

Table 30 l. Series V, no 237—317. Females. Normals, treated with thorium trypan blue.

Days	0—30	31—60	61—90	91—120	121—150	>150	Total
benign. tumour	—	—	—	—	—	—	—
tumour without metastasis	—	1	1	—	—	4	6
tumour with metastases of the lymph nodes only	—	—	—	—	3	5	8
tumour with metastases of the lungs only	—	—	—	1	—	1	2
tumour with metastases in two sites	—	—	1	3	6	7	17
tumour with metastases in more than two sites	—	—	—	—	1	3	4
total	—	1	2	4	10	20	37

Table 31. *Incidence of metastases irrespective of their sites.*

Series	Material treatment	Compared material treatment	Difference
IV, V (No. 237—317 incl.), VI	Non-treated (normal and castrated) females	Thorium trypan blue-treated (normal and castrated) females	26.55 ± 8.78, statistically significant
IV, V (No. 237—317 excl.), VI	Normal (non-treated and treated) females	Castrated (non-treated and treated) females	7.53 ± 5.24, not proved
V, VI, VIII	Non-treated (normal and castrated) males	Treated (normal and castrated) males	7.42 ± 9.24
V, VI, VIII	Normal (non-treated and treated) males	Castrated (non-treated and treated) males	-5.37 ± 5.82, not proved
IV, V, VI, VIII	Non-treated males (normal + castrated) and females (normal + castrated)	Treated males (normal + castrated) and females (normal + castrated)	33.41 ± 11.88, probable and nearly stat. signif.

Table 32. *Incidence of metastases to the regional lymph nodes.*

Series	Material treatment	Compared material treatment	Difference
IV, V (No. 237—317 excl.), VI	Non-treated (normal + castrated) females	Treated (normal + castrated) females	14.59 ± 10.21, not proved
IV, V (No. 237—317 excl.), VI	Normal (non-treated and treated) females*	Castrated (non-treated and treated) females	9.28 ± 5.73, not proved
V, VI, VIII	Non-treated (normal + castrated) males	Treated (normal + castrated) males	-6.10 ± 9.14, not proved
V, VI, VIII	Normal (non-treated + treated	Castrated (non-treated + treated) males	-6.64 ± 5.67, not proved

* No. 237—317 excl.

metastasizing frequency in the non-treated and normal mice respectively is compared, according to the method of computed contingencies to that frequency they would have had if they had been exposed to the same risk as the treated and castrated animals respectively, the results given in the table below are then obtained.

It is apparent from the above table that *treatment with thorium trypan blue causes an inhibition of the metastasizing in the normal castrated females, whereby the difference becomes statistically significant. In the male mice no difference exists.* Castration does not cause any provable inhibition of the metastasizing either in the male or female mice.

a. *Metastasizing to the lymph nodes.*

If one examines the frequency of metastasizing to these lymph nodes, and compares non-treated animals with treated, and normal with castrated, similarly to that made above the according to the standard method of the computed contingencies, the results given in table 32 are obtained.

It can be seen from the above table that neither the treatment with thorium trypan blue nor castration has any provable effect on metastasizing to the regional lymph nodes.

b. *Metastasizing to the lungs.*

Next to the lymph nodes the lungs are the most common region for metastasizing from tumours on the skin of the backs of mice. The incidence of lung metastases during different periods after the origin of the tumour (i. e. at different tumour-ages) appears in the table below.

Table 33. *Incidence of metastases of the lungs at varying periods.* Series IV, V (excl. 237—317), VI, VIII.

days		31–60	61–90	91–120	121–150	>150	Total	Per cent
Norm. ♀	non-tr.	3	7	7	6	10	33	33/75 = 44.00 %
Castr. ♀	non-tr.	1	5	6	3	6	21	21/44 = 47.73 %
	tr.		3	5	7	10	25	25/52 = 48.08 %
V: 237–317	non-tr.		3	2	7	9	21	21/37 = 56.76 %
	tr.		1	6	6	9	22	22/38 = 57.89 %
Norm. ♂	non-tr.		3	11	3	5	22	22/54 = 40.74 %
Castr. ♂	non-tr.		4	9	8	3	24	24/54 = 44.44 %
	tr.	1	2	5	12	6	26	26/58 = 44.83 %

Per cent = % of number of animals with malign. tumour. Norm. = normals. Non. tr. = non-treated. Tr. = treated. Castr. = castrated.

From the above table it appears that lung metastases occur equally as often in normal as castrated mice, and in treated as in non-treated. There is found in the animals in series V. no. 237—317, an increased frequency of lung metastases, probably because the animals were painted with tar (see page 139). This fact is only mentioned in order to show that the treatment with thorium trypan blue does not affect the origin of lung metastases in otherwise non-treated females.

The spontaneously occurring lung adenoma (see page 136) found in 11 animals in series IV, and in 6 in series V and VI, was equally distributed irrespective of castration or treatment with thorium trypan blue, as far as can be judged from this small material.

Table 34. *Incidence of metastases in at least two sites.*

Series	Material treatment	Compared material treatment	Difference
IV, V (No. 237—317 excl.), VI	Non-treated (normal and castrated) females	Treated (normal and castrated) females	3.64 ± 10.06, not proved
IV, V (No. 237—317 excl.), VI	Normal (non-treated and treated) females	Castrated (non-treated and treated) females	0.43 ± 5.17, not proved
V, VI, VIII	Non-treated (normal and castrated) males	Treated (normal and castrated) males	13.44 ± 8.27, not proved
V, VI, VIII	Normal (non-treated and treated) males	Castrated (non-treated and treated) males	5.90 ± 4.81, not proved

Table 35. *Metastases to internal organs.*

	Days	31—60	61—90	91—120	121—150	150	Total
Lung	obs.	6	35	53	52	57	203
	%	1.1	6.6	10.0	9.8	10.8	38.4
Milt	obs.	—	1	3	7	11	22
	%	—	0.2	0.6	1.3	2.1	4.2
Liver	obs.	—	1	2	5	1	9
	%	—	0.2	0.4	0.9	0.2	1.7
Kidney	obs.	—	2	1	4	3	10
	%	—	0.4	0.2	0.8	0.6	1.9
Supra-renal bodies	obs.	—	—	—	2	—	2
	%	—	—	—	0.4	—	0.4
Intestine ...	obs.	—	—	—	—	1	1
	%	—	—	—	—	0.2	0.2

% = per cent of animals with malignant tumour older than 30 days (n = 528) with a tumour-age exceeding 30 days.

c. *Metastasizing to other internal organs.*

As appears from page 139 lung metastases occur more frequently in the animals belonging to series V, than those in series I—IV. In the material which was used to test the effect of lead trypan blue on mouse cancer, metastases to the other internal organs were few. In the material used here considerably more were found, which is perhaps partly because of the longer experiment period, but probably more dependent on the fact of the mice strain's greater susceptibility to develop tumours. Because it appeared that these metastases to the internal organs were equally distributed between normal and castrated mice, between non-treated and treated and between males and females, thus only the incidence of metastasis at different tumour-age is stated in the table given below.

d. *Sarcoma.*

While only two sarcoma were found in series I—IV, a few were found in series V, VI and also in series VIII. As these sarcomae existed to an equally great extent in the animals belonging to series V which were only painted with tar as in the benzpyrene-painted animals, it is possible, but by no means certain, that also the capacity to develop sarcoma or carcinoma may, to a certain degree, be conditioned by heredity. It must be remembered that rats are far more disposed to develop sarcoma than mice whose susceptibility to malignant tumour manifests itself in cancer. Judging from the microscopic preparations, a pure sarcomatous tumour exists only in 13 cases of which merely 7 gave metastases. In the remaining cases it is a question of tumours where 1) sarcoma as well as carcinoma in the tumour, 2) carcinoma in the tumour and sarcoma in one or more of the metastases, 3) sarcoma in the tumour and carcinoma in one or more of the metastases could be found. It is most likely that the majority of these tumours consist of both sarcoma and carcinoma tissue, but accidentally often only one of the tissue types became visible in the microscopic preparations. As only 7 cases of sarcoma which gave sarcoma metastases were found, they have been included in the previous calculation of the latent period before the origin of the tumour, tumourage etc. As far as carcinoma sarcomatodes are concerned there is no reason to distinguish them from carcinoma. The occurrence of sarcoma at different periods appears from the table below.

Table 36. *Sarcoma.* V, VI, VIII.

	Days	31—60	61—90	91—120	121—150	> 150	Total
Norm.	non-tr.	—	1	—	3	2	6
	tr.	—	—	—	—	2	2
Castr.	non-tr.	—	—	—	—	1	1
	tr.	—	1	—	—	3	4
Norm.	non-tr.	—	—	2	3	2	8
	tr.	1	2	—	—	—	2
Castr.	non-tr.	—	1	4	1	—	6
	tr.	—	2	1	2	3	8
Total		1	7	7	9	13	34*

* 5 of these have no metastases.

These tumours have given alternately sarcomatoid or carcinomatoid metastases.

In the below table only sarcomatoid metastases are given.

Table 37. *Sarcomametastases* (resembling sarcoma) are found in following organs.

Days	61—90	91—120	121—150	> 120	Total
Reg. lymph nodes	2	1	5	4	12
Lungs	—	2	2	8	12
Liver	—	2	4	1	7
Milt	—	—	4	7	11
Kidneys	—	—	1	2	3
Total	2	5	16	22	45

3. Analysis of the thorium content in tissue.

The only method to analyse the thorium content in tissue in this investigation is to use a β-sensitive G.-M.-tube. When analysing the ash a little delayed activity could be traced in the liver and tumour, possibly in the kidney and milt but not in the lung and testicles. For this examination the ash from mentioned organs of 20 mice were used. The analysis thus shows a certain concentration of thorium in the tumour and liver.

CHAPTER VII.

General Conclusion and Discussion.

By means of repeated inbreeding, hereditary uniform homozygote mouse strains can now be produced. When examining such strains, it was found that the pure strains could have a different tendency to develop spontaneous mammary tumours, and through inbreeding experiments it could be proved that this tendency is conditioned by hereditary factors. Unfortunately, such uniform strains could not be procured in Sweden when this investigation was begun. The strains used in the experiments are far from uniform. It became evident in the meantime that between these strains there existed a considerable difference as regards the tendency to develop malignant tumours. This difference is statistically significant, and is not only true of the animals' tendency to develop tumours, but also the tumours' distribution tendency. A strain which develops tumours more easily, also more often develops metastases.

Certain minimum doses to induce malignant tumours with carcinogenic substance have, as is known, been previously thoroughly tested. It would therefore be natural if one could, by using larger but not too toxic doses, be able to induce tumours earlier, and obtain an earlier and more pronounced metastasizing tendency. In my experiments, I obtained when using various carcinogenic substances (coal tar and dibenzpyrene) in the same mouse strains, a different length of the

latent period before the tumours' occurrence and a different metastasizing tendency.

In the meantime, it is evident from the above that there should first and foremost exist a hereditary conditioned or inherited factor, which makes the animal more or less sensitive to the carcinogenic irritation. Probably, its intensity comes secondarily as being significant to the tumours' origin and distribution tendency. This is the more probable, as certain kinds of animals can only after large doses of carcinogenic substance develop malignant tumours (e. g. rabbits).

As the female mice in two different strains, used in this investigation, have a higher tumour frequency than the male mice, one ventures to assume that female mice more easily develop skin tumours through carcinogenic influence than the males. It is possible that this difference is because of the difference between the hormonal conditions in female and male mice.

The spontaneously occurring lung adenoma is not so common in my material that any far-reaching conclusions can be drawn. MURPHY & STURM report that lung adenoma becomes considerably more frequent if the mice are painted with coal tar than otherwise. It can be shown from my experiments that the origin of lung adenoma depends considerably on a hereditary conditioned, or an inherited factor. Contrarily, in a strain with a small lung adenoma frequency, one cannot find any difference in the frequency of such, in mice painted with coal tar or dibenzpyrene and in mice which have not been treated at all with carcinogenic agents. Age seems to have a certain influence on the origin of lung adenoma, but probably only to that extent that the animal in time manages to develop tumour adenoma to that degree which it should have in accordance with its hereditary predisposition.

The examination of the toxic and pharmodynamic properties of the preparations used gave the following result. When giving subcutaneous injections to healthy animals, in similar doses as used when treating tumour-bearing animals, lead trypan blue causes a statistically significant shorter length of life in the males. In the females no provable effect is found. Thorium trypan blue causes no shortening of life either in normal or castrated male and female animals. According to GRÜNBERG, lead nitrate 1:1000 affects the sympathicus nerve ends, whereby there arises an inhibition of the internal movements, and a flaccidity of the intestine. Lead trypan blue has, with a concentration corresponding to a lead nitrate solution of 1:240,000, a slight motorial effect. At a subsequent addition of adrenaline, lead trypan blue causes a pronounced stimulating effect on the adrenal inhibition of the intestinal movements. The lead preparation had no, or an uncertain effect on the parasymphaticus and on the muscles of the intestine. Lead trypan blue in the tried dosages had no effect on the uterus. Tests with thorium have not been made previously. It was shown that thorium try-

pan blue in a concentration corresponding to thorium nitrate 1:900,000 gave an increase of tonus of short duration and increase of the amplitude. At a subsequent addition of pilocarpine thorium trypan blue causes an inhibition of the pilocarpine effect, but otherwise no positive effect. Blood pressure tests on rabbits verified previous statements, that thorium should have no effect on the circulation (SOLLMANN). The used preparations thus appeared to be less toxic.

Treatment with lead trypan blue, which was first started when the tumours were positively observed, *gave a statistically significant inhibition of the tumour-growth in mg per day in the test animals.* For both sexes only numerical differences were obtained because the material was too small. Furthermore, *a statistically significant inhibition of the metastasizing was ascertained on the whole as well as to the regional lymph nodes in the males.* In the females there was no provable difference. In the test animals (males and females) a statistically probable and almost significant inhibition of metastasizing to several organs simultaneously was found. In the males the difference was statistically probable, and in the females only numerical. While lead treatment had with certainty an inhibitory influence on the distribution of the tumours in males, any such condition in the females could not be proved statistically.

However, as regards the rate of growth of the tumours, and the more extended metastasizing, such numerical differences in the females are found so that they, when conjoined with those in the males have increased the probability that the treatment has had effect. Therefore, one cannot exclude the possibility that the treatment would have given a positive result on a more comprehensive material of females.

When the treatment of the mice with lead trypan blue was finished, no method was found which was considered as sufficiently sensitive and so making it possible to prove with certainty differences in the lead content in the various tissues in the small amounts used here. Since this method has been essentially improved, a semiquantitative microspectroscopic-analytical determination of the lead content in the tumour tissues and liver in one mouse has been made. According to FLURY's work, the liver contains comparatively more lead than the other internal organs. It is therefore remarkable that when analysing, approximately twice as much lead is found in the tumour as in the liver. In view of the fact that the heavy ions are known for their regular distribution in the organism, the result of this one examination can possibly be generalised, and it can, furthermore, be assumed that a certain tendency to accumulate lead in the tumour tissues existed. At the obduction, a macroscopic staining with lead trypan blue was found most in the kidneys, less in the liver, milt and testicles, whereas a noticeable staining of the tumour could not be found in any of the cases.

The inhibition of the tumours' rate of growth may possibly be caused

by an accumulation of lead in the tumour tissue, whereby the lead should have an inhibitory effect on the rate of growth of the tumour cells. As regards the metastasizing, it can possibly be thought that leadtrypan blue similarly to other colouring agents can firstly be accumulated in the lymph nodes and other reticulo-endothelial organs, and here have a local inhibitory effect on the function of the metastastic tumour cells. As, however, the testicles were especially coloured, and as the analyses showed a concentration of lead in the testicles it is probable that the lead preparation had some effect on the hormone production in the testicles, which could secondarily have some effect on the tumour growth and distribution. The fact that lead trypan blue in not stained microscopical slides is seen in the interstitial tissue speaks in favour of this.

As regards the chemical constitution of trypan blue it is not probable that leuko-compounds can be formed. It is possible that lead trypan blue is decomposed in the tumours so that the lead can remain without macroscopically colouring the tumour. Contrarily the lead content should for example in the liver correspond the amount of lead trypan blue which has already macroscopically stained the liver.

In the castration experiments, the operation was performed when the animals were $2\,{}^{1}/_{2}$—3 months old, a few weeks before the treatment with carcinogenic agents began. By means of a very long observation time (more than 40 weeks) it was proved that castration does not in this material influence the tumour frequency in proportion to the number of painted animals, either in males or females. On the other hand, *castration caused a statistically significant later occurrence of tumours in males*. There was no provable difference in the females, although the results give a similar indication as in the males. Castration may possibly though it is not probable have caused an inhibition of the general development in the males, which is not so pronounced in the females.

After the tumours had appeared there was no difference between the castrated and non-castrated animals as regards the rate of growth or metastasizing tendency. *The castrated males and females had a certain tendency to live longer than the normal males and females*, and this difference in the older ones was statistically probable or significant. If the castrated males and normal males are compared, the same tendency is found though less pronounced. Only in the females there is no provable difference.

Previously, only one investigation of the effect of castration on tar cancer in mice has been carried out on a satisfactorily large material (Katz). He believed that he found an inhibited occurrence of tumours in the castrated female mice, and also a decreased metastasizing frequency. According to his report it seems as if the normal animals ("Grundversuch") were not treated simultaneously with the castrated, thus it is unknown whether they belonged to the same strain. Besides

he generally only had 28 weeks observation period (in some experiments 32 weeks) after he had started painting; this will influence the obtained figures for the tumour frequency. In my material some of the tumours occured after 40 weeks. It is maintained in the description of the method, that in all cases certain organs were examined microscopically. As the regional lymph nodes are not mentioned, but he states that metastases are most common in the lungs, it is doubtful whether the information of the metastasizing frequency is quite correct. It is impossible to estimate the metastasizing frequency with any certainty without a serial section of the lymph nodes. As both in VANNFÄLT's and my investigation, tumours most frequently metastasize to the regional lymph nodes, it is conceivable that the lymph nodes are the most usual metastasizing site for the tumour in mice. If, in view of these remarks, the results of KATZ are examined, they become uncertain. Comparing these to my experiments with a longer observation period and a considerably larger material, no probable effect of castration on the females could be determined. In the males there was an inhibition of the tumours' development in the form of delayed tumour formation which has not been described previously.

Treatment with thorium trypan blue, given to both normal and castrated animals, first began when the animals developed a positively observed tumour. Thus it can only have influence on the tumours' development from this time. It became apparent that the treatment caused a longer length of life in the castrated females in comparison to non-treated, castrated females, whereby the difference is only statistically probable. No provable difference is found in the males.

Castration seems to have had no effect as regards the rate of growth of the tumours.

The treatment with thorium trypan blue caused a statistically significant inhibition of the metastasizing in the females, no difference was found in the males. Contrarily, when examining the metastases to the regional lymph nodes or to the internal organs, only a numerical difference could be found which showed the same tendency. If consideration is taken to the fact that the treatment caused a statistically probable longer tumour-age in the castrated females as compared to the non-treated castrated females, it can perhaps explain the fact that no difference was found as regards metastasizing to the lymph nodes and internal organs. Tumours probably give more and more metastases at higher tumour-ages. It is of course possible that if the treated animals had been killed at the same age as when the non-treated died, a statistically proved difference in the metastasizing could have been obtained instead of this indicated difference in the length of life. The remarkable fact is that we did not obtain any difference between the males.

Treatment with thorium trypan blue seemed to have no inhibitory effect on the tumour-growth. No provable difference between the

various groups, between the normal and castrated animals was found. When conjoining the material consisting of castrated males and females in series V and VI, and the females 237—317 in series V which belonged to the same strain, and then compared the treated with the non-treated it was found that the non-treated mice had a numerically larger tumour-growth in mg. per day; the difference becomes 8.2 ± 4.5 mg. The treated animals had a higher average tumour-age than the non-treated; the difference becomes 12.8 ± 4.8 days and thus probable and nearly statistically significant. Considering the higher average tumour-age and the numerically lower average tumour-weight, it may be assumed that *at the same tumour-age in the treated and non-treated animals it should have been possible to prove a retardation of the rate of growth of the tumours.*

Thus, it is probable that in a larger material comprising test and control animals it should have been possible to prove a retarding of the rate of growth of the tumours as well as the metastasizing tendency in females.

When analysing the tumour tissue there could be proved a certain concentration of thorium in it. It is possible that thorium as a heavy ion could inhibit the rate of growth of the cells. Similarly could thorium when stored in the lymph nodes and other reticulo-endothelial organs inhibit the metastasizing. As this could only be proved in the females it is more probable that the results of the treatment with thorium trypan blue could be due to the fact that the females could be more sensitive to the weak radio active effect which this preparation possesses.

In the survey of the literature, the mentioned cancer-inducing effect of thorotrast could not be shown with the doses used here.

The treatment with leadtrypan blue had thus an effect mainly on the males, and thoriumtrypan blue mainly on the females. This fact together with the retarded occurrence of the tumours in the castrated males shows that *the hormonal factors are of significance to tumours which do not belong to organs directly dependent on the sexual hormones.*

Summarizing, it has been proved in this investigation that treatment with lead trypan blue inhibited the tumour growth and metastasizing in males especially, possibly due to the concentration of lead trypan blue in the testicles. The treatment with thorium trypan blue inhibited the tumour growth and metastasizing in female mice especially. This result has been obtained by using mainly nontoxic doses. Castration caused a retarded occurrence of the tumours in male mice. The results are such, that it is possible that by using lead and thorium in eventually larger doses on a larger material an effective treatment can be obtained in both sexes. The difference in the results between the sexes, and also the results of castration enables the hormonal factors appertaining to sex to have a significant influence on the development and metastasizing even of those tumours which occur at sites which á priori are not dependent on the sex hormones. Earlier, such a condition has only been proved in spontaneous mammary tumours in certain mouse strains.

CHAPTER VIII.

Summary.

The survey of the literature reveals that the problems concerning the influence of metal salts on malignant tumours cannot be considered as being finally elucidated. This is not only true of the ability of the heavy ions to affect inhibitorily the enzyme processes in the tumour tissues, but also of the possibility of using relatively weak radio-active isotopes the radiation effect of which could have some influence on the growth and distribution tendency of the tumours. Furthermore, satisfactory investigations are missing as to the effect of hormonal factors on the tumours outside the regions directly influenced by the sex hormones. In the latter, the response of tumours to treatment with hormones is also still not sufficiently clear.

The present investigation has been carried out through experiments on a mouse material comprising almost 800 tumour-bearing mice. The problems that have been especially examined are firstly the influence of lead trypan blue, the influence of weak radio-active thorium and the influence of castration on mouse tumour. The most important results are the following:

1) treatment with lead trypan blue gives a statistically probable and almost significant inhibition of the rate of growth of tumours which is probably caused by a concentration of lead in the tumour tissue, according to some few determinations. No difference in mortality as compared to nontreated animals could be stablished.

2) treatment with lead trypan blue gives a statistically significant inhibition on metastasizing in males but has no certain influence on females. According to experiments the result depends possibly on a concentration of lead in the testicles.

3) castration before painting with tar and dibenzpyrene gives a statistically significant retardation of the occurrence of tumours in males, but no proveable difference in females. When the tumour had developed, no proveable difference in their growth and distribution tendency could be proved.

4) treatment with thorium trypan blue gives a statistically significant longer life in animals after the origin of the tumour and probably also an inhibition on the rate of growth of the tumours. A certain concentration of thorium in the tumour tissue was proved which should explain an inhibition of the rate of growth.

5) treatment with thorium trypan blue gives a statistically significant inhibition of the metastasizing in females inspite of the longer tumour-age in the treated animals. Since corresponding results in males are not found this may possibly depend on a greater sensitiveness in the males to the radio-active influence of the preparation.

Summarizing, it has thus been proved that the employed mouse tumour, inspite of its position outside the regions dependent upon the sex hormones, is nevertheless easily hormonally influenced.

CHAPTER IX.

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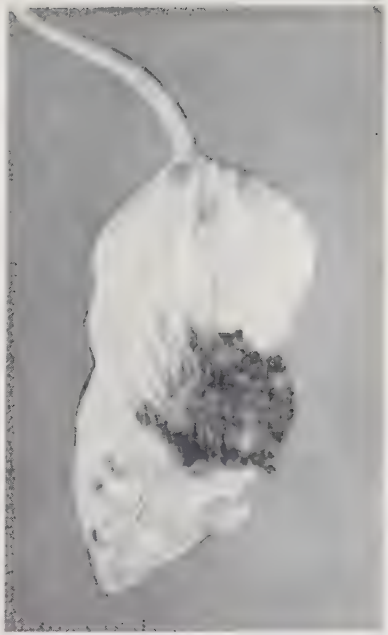


Fig. 5 and 6. Mice with cancerous tar tumours.

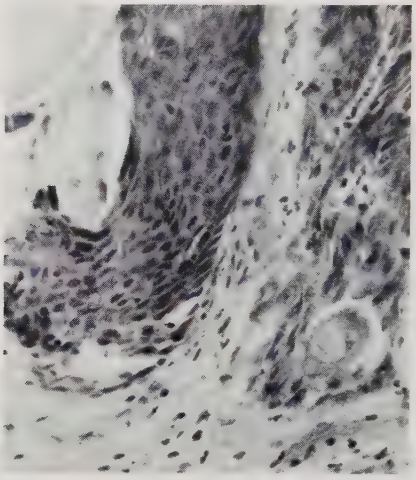


Fig. 7. The commencement of infiltration of the subcutaneous connective tissue.

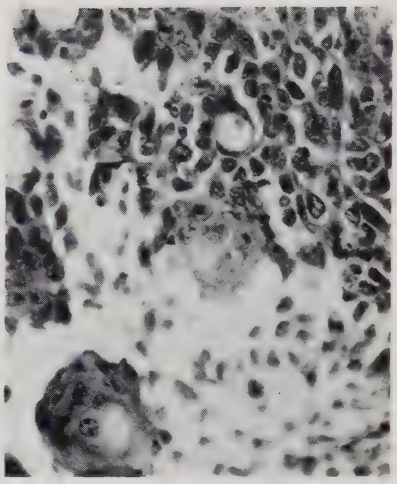


Fig. 8. More advanced infiltration of the subcutaneous connective tissue.

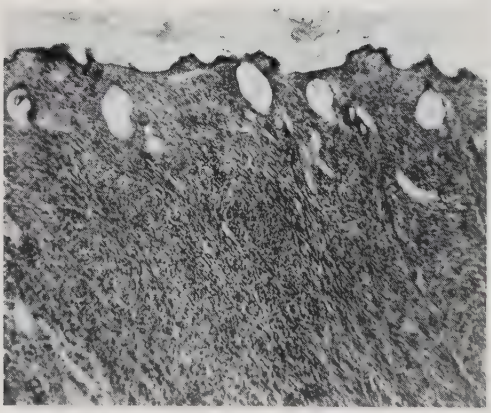


Fig. 9. Sarcomatous tumour growing in under the epidermis.

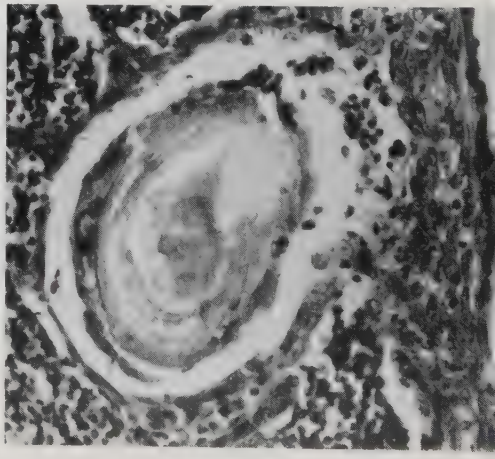


Fig. 10. Early cancroid metastasis in a lymph nod

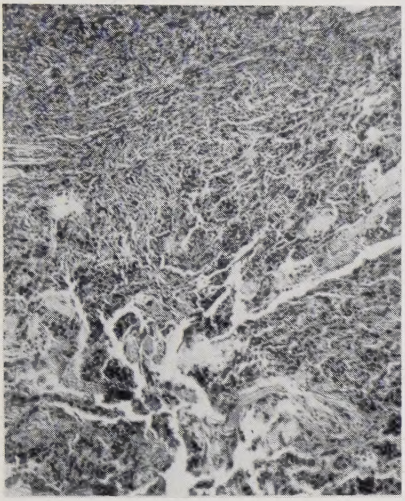


Fig. 11. Tumour showing the polymorph structure Upper: Sarcomatous tissue. Middle: Cancer simplex. Lower: Cancroid.

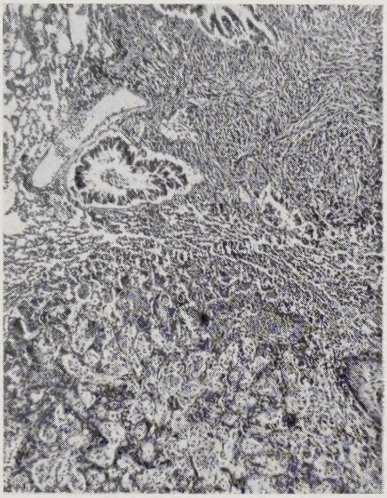
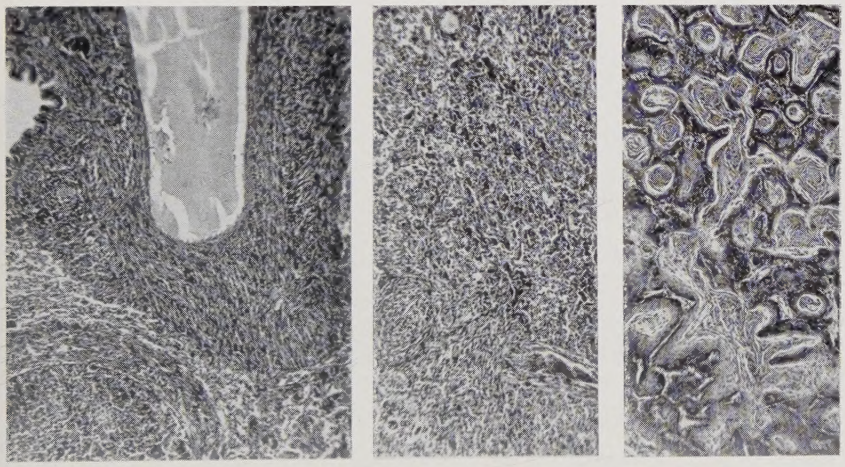
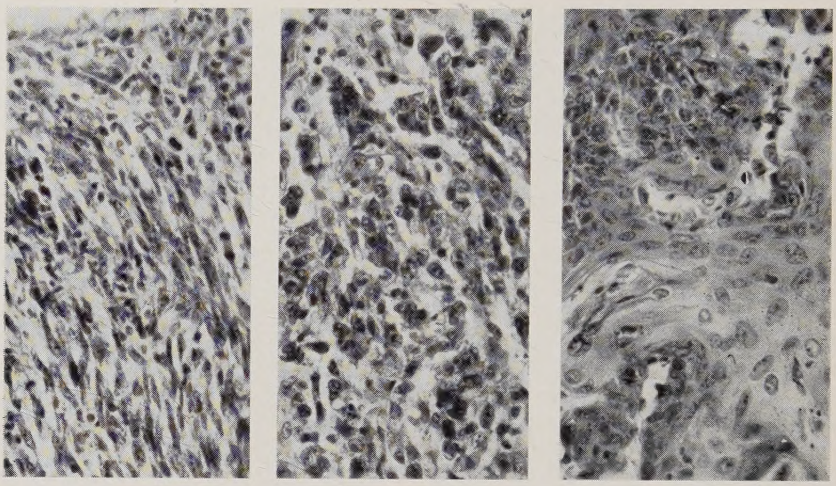


Fig. 12. Metastasis of the same structure in the lung.



a b c

Fig. 13. Metastasis in the lung, the figures showing different parts of the same slide, in a) sarcomatous tumour growing around and in the vessels, b) cancer simplex, c) cancroid.



a b c

Fig. 14. The same tumour (Fig. 13) enlarged.

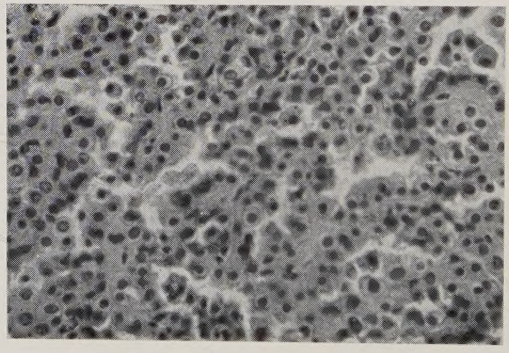


Fig. 15. Showing the structure of a lung adenoma, which is indisputably different to a lung metastasis.

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